

Management of Adrenal Masses in Children and Adults



Electron Kebebew
Editor

 Springer

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I would like to dedicate this book to my wife Tida, and sons Ras and Ezana.

I would also like to thank all my teachers, mentors, and colleagues for their support.

Preface

Adrenal neoplasms are common and are often discovered incidentally. Virtually every medical provider will encounter a patient with an adrenal neoplasm during their training and clinical practice. Given the aging population, the number of patients found to have an adrenal neoplasm will continue to increase.

The field of adrenal neoplasms has been transformed due to significant developments in the last decade. There have been significant advances in our knowledge of adrenal neoplasms include the identification of genetic changes that result in these tumors, and genotype–phenotype associations that have emerged and that have significant clinical implications in the management of these tumors, including screening of at-risk family members. The type of treatment selected, improved high resolution anatomic and functional imaging studies, advances in biochemical diagnostic tests to distinguish between functioning and nonfunctioning tumors, and the application of advanced surgical technology and techniques have improved patient outcome.

This unique textbook is focused on adrenal neoplasms and written by a multidisciplinary team of experts. It provides a comprehensive, state-of-the art view of this field, and will serve as a valuable resource for medical students, clinicians, and researchers. The book reviews new data about common genetic syndromes that impact management strategies, imaging, biochemical evaluation to optimize treatment, and new and emerging approaches to the treatment of adrenal neoplasms.

We hope this textbook will serve as a useful resource for all providers dealing with, and interested in these common but challenging tumor.

Bethesda, MD, USA

Electron Kebebew

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Epidemiology of Adrenal Neoplasms

Pavel J. Nockel and Electron Kebebew

Introduction

Adrenal neoplasms are common and represent a growing clinical problem that requires appropriate clinical work up and management to reduce morbidity and mortality. One of the main reasons that the incidence and prevalence of adrenal neoplasms has increased is as a result of incidentally detected tumors, referred to adrenal incidentalomas. The term adrenal incidentaloma refers to an adrenal neoplasm that is detected on diagnostic imaging studies for nonadrenal clinical disorders and that measures more than 1 cm. These clinically inapparent adrenal neoplasms have become common clinical findings because of improvements in imaging technique and technology, and the increasing use of imaging studies in clinical practice. Although the vast majority of adrenal incidentalomas are nonfunctioning, benign

tumors, there is growing evidence that the natural history of such tumors may have important clinical consequences and requires appropriate follow up and treatment [1, 2]. Patients with adrenal neoplasms may also present with signs and symptoms associated with hormonal hypersecretion and due to local symptoms from tumor mass effect.

The increasing incidence and prevalence of adrenal neoplasms is reflected in the medical literature as there has been an increase in studies published concerning adrenal neoplasms/incidentalomas over the last two decades, as well as, an increase in the number of adrenalectomies being performed for adrenal neoplasms (Fig. 1.1) [3, 4].

The evaluation and management of adrenal incidentalomas is centered on determining whether the tumors are causing hormonal hypersecretion (functioning) and/or are malignant (primary or metastasis). The differential diagnosis for an adrenal neoplasm is broad and depends on patient age, clinical presentation, imaging characteristics, and biochemical testing results (Table 1.1). The differential diagnosis for adrenal incidentalomas includes primary adrenal tumor, adrenal metastasis, and tumors originating from adjacent tissues and organs, such as the stomach, tail of the pancreas, and retroperitoneum (lymphomas, sarcomas). Infrequently, adrenal lesions such as

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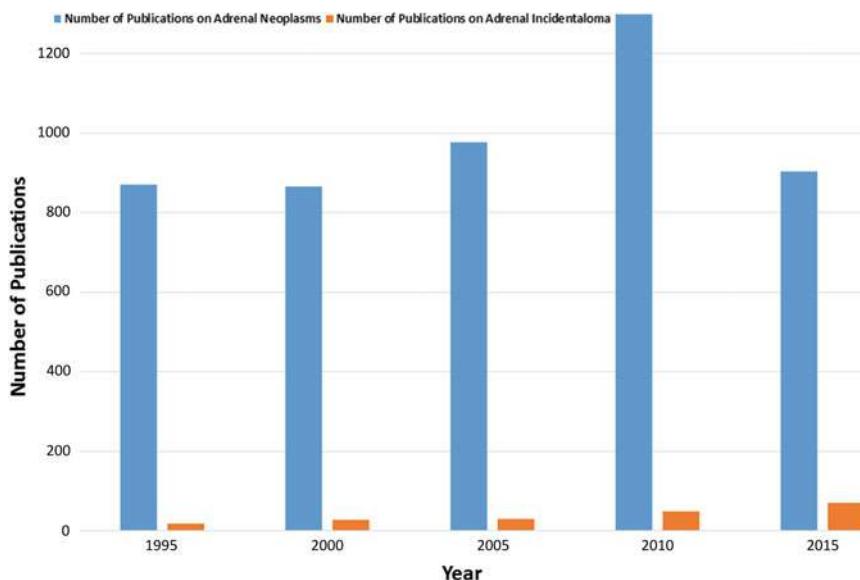


Fig. 1.1 Trend on literature publications focused on adrenal neoplasms and incidentaloma. A keyword search using “adrenal neoplasms” and “adrenal incidentaloma” was performed in PubMed by year of publication

Table 1.1 Types of adrenal neoplasms

Primary adrenal neoplasm
<i>Nonfunctioning</i>
Cortical adenoma/carcinoma
Cyst
Myelolipoma
Ganglioneuroma
Hemorrhage/hematoma
<i>Functioning</i>
Cortical adenoma/carcinoma
Hypercortisolism
Hyperaldosteronism
Sex hormone excess
Mixed steroid hormone excess
Pheochromocytoma
Metastasis
Lung
Kidney
Breast
Gastrointestinal
Melanoma
Other primary tumors which can masquerade as adrenal neoplasm
Lymphoma
Pancreatic tail mass
Extra-adrenal paraganglioma
Gastric tumor

adrenal cysts, hematoma/hemorrhage, lipoma, or granulomas may present as adrenal incidentalomas.

Many adrenal neoplasms can be diagnosed by their pathognomonic imaging characteristics on computed tomography (CT) and/or magnetic resonance imaging (MRI). For example, adrenal cysts usually can be diagnosed by characteristic CT scan criteria alone. The cysts are commonly round, smooth, and have a homogenous water density. Adrenal hemorrhage may also masquerade as adrenal neoplasms, but the diagnosis can be established based on the history and CT scan findings. Patients with adrenal hemorrhage commonly have a history of sepsis, trauma, or anticoagulation treatment. Bilateral adrenal hemorrhage may occur in some patients who have sepsis or are on anticoagulation therapy. Adrenal hemorrhage or hematoma usually has a round-oval and inhomogeneous appearance on CT scan. The periadrenal fat and soft tissue may also show infiltration. These findings usually resolve during follow-up imaging studies. Adrenal myelolipomas have a characteristic fat density, are benign, and consist of fat and mature myeloid tissue. Adrenal myelolipomas may result in locoregional symptoms if they are large. Some patients may

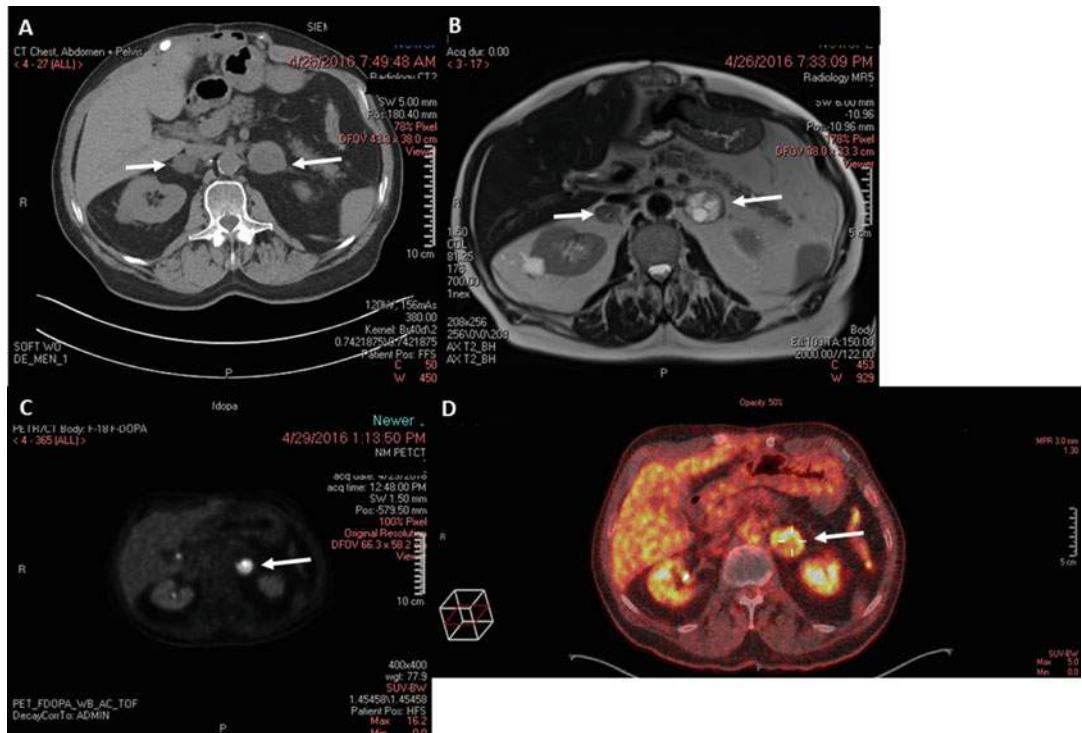


Fig. 1.2 Multimodal imaging results in a patient found to have bilateral adrenal masses in the setting of multiple endocrine neoplasia type 1 and with biochemical evidence of pheochromocytoma. **(a)** Computed tomography scan shows bilateral adrenal neoplasms. The right adrenal tumor has a lower Hounsfield unit than the left adrenal mass. **(b)** Magnetic resonance imaging shows bilateral adrenal masses but the left adrenal mass enhances on T2-weighted image which is suggestive of a pheochromocytoma. **(c)** F-18F DOPA imaging shows uptake in the left adrenal gland only, which is consistent with a pheochromocytoma. **(d)** 18 F FDG PET/CT imaging show increased uptake in the left adrenal gland but not right adrenal mass which is also consistent with a pheochromocytoma in the left adrenal gland. The right adrenal tumor was consistent with a nonfunctioning adrenocortical tumor. The left adrenal tumor was consistent with a pheochromocytoma on pathologic examination

cytoma. **(c)** F-18F DOPA imaging shows uptake in the left adrenal gland only, which is consistent with a pheochromocytoma. **(d)** 18 F FDG PET/CT imaging show increased uptake in the left adrenal gland but not right adrenal mass which is also consistent with a pheochromocytoma in the left adrenal gland. The right adrenal tumor was consistent with a nonfunctioning adrenocortical tumor. The left adrenal tumor was consistent with a pheochromocytoma on pathologic examination

have bilateral adrenal masses discovered on imaging studies. These bilateral adrenal incidentalomas can be due to infectious granulomas (tuberculosis, fungal, meningioccus), lymphoproliferative disease, or amyloidosis. Bilateral adrenal masses also may be due to hereditary pheochromocytomas (adrenal medullary hyperplasia) or macronodular and micronodular cortical hyperplasia (Fig. 1.2).

In this chapter, the incidence and prevalence of adrenal neoplasms in association with specific clinical presentation is discussed. The specific types of tumors that require clinical evaluation in children and adults are discussed in detail in other chapters of this textbook focused on adrenal neoplasms. The management of neuroblastomas

is not discussed in this book as these tumors usually occur in infancy. Lastly, known risk factors for adrenal neoplasms are discussed.

Adrenal Neoplasms Detected at Autopsy

Autopsy studies that have evaluated the prevalence of adrenal neoplasms provide useful information in understanding the clinical significance of adrenal incidentalomas. The prevalence of adrenal neoplasm in autopsy studies has been reported to be 0.34–8.7% in patients who died of other causes, with an average prevalence of 1% (Table 1.2) [5–20]. The wide range in prevalence

Table 1.2 Rate of adrenal incidentaloma in autopsy studies [5, 6]

Author, year, reference	Sample size of entire study cohort	Study design	Rate of adrenal neoplasm/ incidentaloma (%)	Study population gender (MF)	Median/mean age of study cohort (age range)	Study cohort with history of malignancy (yes/no/NA)
Rinehart 1941 [7]	100	NA	3/100(3 %)	NA	NA	NA
Russi 1945 [8]	9000	NA	131/9000(1.45 %)	71/60	60–69(9–89)	NA
Commons 1948 [9]	7437	Prospective	216/7437(2.86 %)	130/86	60–70(20–90)	NA
Schroeder 1953 [10]	4000	NA	55/4000(1.38 %)	NA	NA	NA
Devenyi 1967 [11]	5120	NA	185/5120(3.5 %)	100/85	61–70(31–80)	NA
Kokko 1967 [12]	2000	NA	21/2000(1.05 %)	NA	NA	NA
Hedeland 1968 [13]	739	Prospective	64/739(8.7 %)	36/28	NA	NA
Yamada 1969 [14]	948	NA	51/948(5.4 %)	NA	NA	NA
Granger 1970 [15]	2425	NA	61/2425(2.5 %)	35/26	59.7(standard deviation 12.8)	NA
Russell 1972 [16]	35,000	NA	690/35,000(1.97 %)	NA	NA	NA
Abecassis 1985 [17]	988	Retrospective	19/988(1.9 %)	NA	Yes	NA
Meagher 1988 [18]	2951	Retrospective	149/2951(5 %)	NA	No	NA
Kawano 1989 [19]	153,000	NA	519/153,000(0.34 %)	NA	NA	NA
Reinhard 1996 [20]	498	NA	25/498(5 %)	NA	NA	NA
Total	224,206		2189/224,206 (0.97 %)			

NA not applicable

is due to study cohort age distribution, causes of death, level of pathologic examination, and the presence of risk factors for adrenal neoplasms. The prevalence of adrenal neoplasms is also higher in individuals with obesity, diabetes, and hypertension [13, 21]. In most autopsy studies, the adrenal neoplasms were consistent with a cortical adenoma.

Adrenal Neoplasms Detected on Imaging Studies (Adrenal Incidentaloma)

Most adrenal neoplasms are detected as an adrenal incidentalomas and are commonly due to nonfunctioning cortical adenomas. Adrenal incidentalomas are found in approximately 1 % of individuals undergoing abdominal imaging studies (Table 1.3) [17, 22–32]. The incidence and prevalence of non-functioning adrenal incidentaloma depends on patient age. For example, adrenal incidentalomas occur in less than 1 % of patients <30 years old but in more than 7 % of patients 70 years and older. The incidence and prevalence is highest in older patients with a prevalence of up to 8.7% reported (see Table 1.3). Another important determinant of the incidence and prevalence of adrenal incidentaloma is the tumor size used to define this entity. While generally a tumor at least larger than 1 cm is used in most studies, if tumors less than 1 cm are considered higher prevalence rates would be observed. The prevalence of adrenal incidentaloma also depends on the sensitivity of the imaging study. Furthermore, adrenal incidentaloma are more commonly discovered in patients with a history of cancer, but when discovered during staging work up in cancer patients they should not be referred to as adrenal incidentalomas. Although more adrenal incidentalomas have been reported in women, this is likely due to more common use of imaging studies in women.

An accurate estimate of the incidence of adrenal neoplasms in population-based studies is lacking. However, based on the relative overall prevalence of adrenal incidentalomas of 1% and approximately 80 million CT scans being performed

annually in the United States, it is estimated that the incidence of adrenal neoplasms/incidentalomas is 2–8 cases per 100,000 persons a year.

Incidence and Prevalence of Functional Adrenal Neoplasms

Pheochromocytoma

Pheochromocytoma hypersecrete catecholamine and metabolites and are rare adrenal tumors and occur in the adrenal medulla. They originate from the neural crest cells. Pheochromocytomas usually are symptomatic and present with clinical symptoms associated with catecholamine excess (hypertension that is sustained or paroxysmal, headache, episodic profuse sweating, palpitations, pallor, apprehension, and/or anxiety). Pheochromocytoma occurs in approximately 0.2 % of patients with hypertension. The estimated annual incidence of pheochromocytoma is 0.1–0.6 cases per 100,000 persons a year. The average age of pheochromocytoma development is in the fifth-to-sixth decade of life but also depends on the catecholamine profile and whether the patient has an inherited genetic syndrome, the age of onset can be as early as 5 years of age [33, 34].

Adrenal Cushing Syndrome

Adrenal Cushing syndrome has a predominance in women with a female-to-male ratio of 3:1. The estimated annual incidence of Cushing syndrome is 2–5 cases per million people, and the prevalence is 39–79 cases per million person [21, 24, 33, 35]. Approximately 10 % of adrenal Cushing syndrome occur in children with most being part of an inherited syndrome [35]. Adrenocortical adenomas account for approximately 80 % of adrenal Cushing syndrome and 15 % are due to adrenocortical carcinoma [17, 25].

Among the less common causes of ACTH-independent Cushing syndrome are the familial and sporadic forms of bilateral macronodular and micronodular adrenal hyperplasia with its

Table 1.3 Prevalence of adrenal neoplasms in computer tomography imaging studies [5, 6, 9, 39]

Author, year, reference	Sample size of entire study cohort	Study design	Rate of adrenal neoplasm/ incidentaloma (%)	Study population Gender (M/F)	Median/mean age of study cohort (age range)	Study cohort with history of malignancy (yes/no/NA)
Glazer 1982 [22]	2200	Retrospective	16/2200(0.7%)	7/9	57(40–76)	NA
Prinz 1982 [23]	1423	Retrospective	9/1423(0.6%)	5/4	58(41–73)	NA
Abecassis 1985 [17]	1459	Retrospective	19/1459(1.3%)	10/9	60.8(37–83)	No
Belldegrun 1986 [24]	12,000	NA	84/12,000(0.7%)	NA	NA	NA
Herrera 1991 [25]	61,054	NA	342/61,054(0.5%)	136/206	62	No
Caplan 1994 [26]	1779	Retrospective	26/1368 (1.9%)	9/17	66(36–86)	No
Bovio 2006 [27]	512	Retrospective	22/512(4.2%)	17/5	58(50–79)	No
Song 2007 [28]	3307	Retrospective	290/3307(8.7%)	94/196	64(20–93)	No
Song 2008 [29]	63,004	Retrospective	973/63,004(1.5%)	406/567	64(19–100)	No
Muth 2011 [30]	3801	Prospective	226/3801(5.9%)	85/141	67	No
Davenport 2011 [31]	3099	Retrospective	37/3099(1.1%)	20/17	68(45–92)	No
Bhat 2015 [32]	4132	Prospective	213/4132 (5.15%)	136/77	NA	No
Total	157,770		2257 (1.4%)	929(43%)/1249(57%)	62.48	

NA not applicable

pigmented variant also referred to as primary pigmented nodular adrenocortical disease [3, 22, 35].

In children before the age of 7 years, the primary adrenal causes of Cushing syndrome including adenoma, carcinoma, bilateral macronodular, and primary pigmented nodular adrenocortical disease are most common [24]. Unilateral adrenal tumors presenting with Cushing syndrome in young children (under age of 5 years) are usually malignant (more than 70 % of cases) [24]. In children with a known family history of hereditary cancer syndromes such as Carney complex, multiple endocrine neoplasia type 1, and hereditary leiomyomatosis/renal cancer syndrome, endogenous hypercortisolism may be the first manifestation.[36].

Adrenocortical Carcinoma

Adrenocortical carcinoma is a rare but fatal malignancy. The estimated incidence is 0.7–2.0 cases per million persons each year [37–39]. Adrenocortical carcinoma has a bimodal age distribution at presentation with most cases of childhood adrenocortical carcinomas being due to germline *TP53* mutations, especially in Brazil. There are no precise estimates of adrenocortical carcinoma prevalence, but the prevalence of adrenocortical carcinoma in patients undergoing an adrenalectomy for adrenal neoplasms can range from <1 % to up to 75 % depending on the study cohort, indications, and tumor size [40]. In patients with non-functioning adrenal neoplasms and inconclusive imaging features, the risk of adrenocortical carcinoma is an important diagnosis to exclude as it is often incurable if not completely resectable and is associated with high mortality.

Primary Hyperaldosteronism

Primary hyperaldosteronism commonly present in the third to sixth decades of life and rarely occurs in children unless they have a familial form of primary hyperaldosteronism [15, 16]. The prevalence of primary hyperaldosteronism is

variable depending upon the study population. In the Framingham Offspring study, in which 3326 adults with untreated hypertension were screened for primary hyperaldosteronism, 7.6 % of men and 26 % of women were found to have primary hyperaldosteronism [17]. In a study from Italy, Rossi and colleagues found 11.2 % of 1,125 patients with hypertension had biochemical evidence of primary hyperaldosteronism [10]. Therefore, the prevalence of primary hyperaldosteronism is high in patients with hypertension (10 %), and even higher in those with hypertension resistant to treatment (20 %) [18, 19].

Risk Factors for Adrenal Neoplasms

The main risk factor for an adrenal neoplasm is a family history of adrenocortical tumors and pheochromocytoma. An isolated family history of adrenocortical carcinoma due to germline mutation in *TP53*, Li–Fraumeni syndrome, Beckwith–Wiedemann syndrome, multiple endocrine neoplasia type 1, congenital adrenal hyperplasia, familial polyposis coli, and β-catenin mutations are associated with a higher risk of adrenocortical tumors (benign and malignant). Although patients with a family history of primary hyperaldosteronism are also at risk of adrenal neoplasm, most of these patients present with severe hypertension that prompts biochemical screening testing and routine imaging to detect an adrenal neoplasm is not indicated.

Patients with a family history of pheochromocytoma/paraganglioma are also at risk of developing adrenal neoplasms. These inherited syndromes include von Hippel–Lindau syndrome, multiple endocrine neoplasia 2, neurofibromatosis type 1, and familial paraganglioma/pheochromocytoma syndrome (due to mutations in succinate dehydrogenase B [*SDHB*] and D [*SDHD*] subunits). More than 14 genes with germline or somatic mutations have been associated with pheochromocytoma, so patients with a known germline mutation and or family history of pheochromocytomas should be screened for tumor development.

While no environmental factors have been associated with the risk of developing an adrenal neoplasm, several clinical features and characteristic have been associated with higher rates of adrenal incidentaloma/neoplasms. These include patients with sleep apnea, hypertension, diabetes, obesity, and osteoporosis.

Summary

Adrenal neoplasms are common and the rate of incidentally detected adrenal neoplasm is increasing. Although the majority of adrenal neoplasms discovered incidentally are benign and nonfunctioning, proper evaluation to exclude a functioning tumor and malignant tumor is important to reduce morbidity and mortality. Long-term follow up of adrenal neoplasms is imperative as a subset of patients may require intervention. The main risk factor for developing adrenal neoplasms is a family history and should prompt clinical evaluation and intervention when indicated.

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Ranran Zhang and Ricardo V. Lloyd

Development, Anatomy, and Physiology

Development of the Adrenal Glands

The adrenals are bilateral glands located on the superomedial aspects of the kidney. Two different endocrine tissues types, including the cortex and the medulla, are intimately associated with each other in the adrenal glands. Because of this intimate relationship, hormones from one portion of the adrenal influence other parts of the glands. For example, the synthesis of epinephrine from norepinephrine in the medulla is influenced by the glucocorticoids produced and secreted in the adrenal cortex. The cortex can be detected at 5–6 weeks of gestation in the 9-mm embryo stage. Cortical cells can be detected as proliferations of primitive coelomic cells arising from the peritoneum in the dorsal mesentery [1, 2]. By 8 weeks of gestation the cortical cells appear as a distinct unit with a fibrous capsule separated from the adjacent mesothelium. The fetal cortex is prominent during gestation and forms a distinct fetal zone that is larger than the adjacent definitive cortex. The fetal zone comprises around 75 % of

the cortex at birth. The fetal zone involutes during the first 6 months after birth [2]. There are three zones in the adult adrenal gland including the zona glomerulosa, fasciculate, and reticularis. The distinct zones of the cortex are fully developed by the time of puberty. The paraganglionic tissues within the adrenal and in the abdomen arise from the neural crest [3, 4]. The primitive sympathetic cells and nerve fibers invade the paravertebral and paravertebral sympathetic tissue and extend into the adrenal cortex around weeks 5–6 weeks of gestation. The primitive sympathetic cells initially appear as nodular aggregates in the cortex. Chromaffin cells can be identified by 7–8 weeks of gestation. The primitive sympathetic cells peak around 17 and 20 weeks and then the nodules of cells decline subsequently. However, groups of nodular primitive sympathetic cells may persist after birth and into early infancy. The extra-adrenal chromaffin cells involute during the latter part of fetal life and continue to involute after birth [3, 4].

Anatomy and Physiology

Each adult adrenal weighs between 4 and 5 g after the peri-adrenal adipose tissue is carefully removed [5]. Patients with chronic illnesses may have larger glands resulting from prolonged stimulation of the glands secondary to stress [6]. The right adrenal is usually pyramidal in shape

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while the left gland is crescentic or lunate in shape. Cut sections of a freshly prepared adrenal usually reveal a bright yellow outer cortex while the inner cortex is tan. The adrenal cortex in adults constitutes around 90% of the weight of the gland [7]. The zona fasciculata comprises about 70–80% of the cortical volume while the glomerulosa makes up about 15% of the volume. The zona fasciculata and reticularis synthesizes glucocorticoids and sex steroids while the glomerulosa synthesizes mineralocorticoids and is less responsive to adrenocorticotrophic hormone (ACTH) than the other two zones. The zona reticularis contains cells with eosinophilic cytoplasm, which can synthesize both glucocorticoids and sex steroids. Ultrastructural examination of the cortex shows cells with abundant smooth endoplasmic reticulum and mitochondria with bulbous cristae, features of steroid-producing cells. The secretion of aldosterone by the zona glomerulosa is regulated by the renin–angiotensin system, while the other two zones are regulated by ACTH. The adrenal medulla in adults occupies about 10% of the gland volume. Most of the medulla lies within the head of the gland with a smaller portion of medullary tissue within the body. The tail of the adrenal is usually devoid of medullary tissue in the normal gland, but medullary tissue may be present in this region in patients with medullary hyperplasia [8, 9].

Adrenocortical Masses

Adrenal cortical masses may be caused by hyperplasia of the cortex, by primary benign and malignant adrenocortical neoplasms and by other lesions such as cysts of the adrenals, myelolipomas, and metastatic tumors to the adrenal glands.

Adrenocortical Hyperplasia

Hyperplasia Associated with Pituitary ACTH and Hypothalamic CRH Overproduction

An increase in cortical mass may be associated with stimulation of the adrenal cortex by ACTH from the anterior pituitary or from ectopic

sources. Excessive production of corticotropin-releasing hormone (CRH) from the hypothalamus or ACTH from the pituitary can lead to hyperplasia of the adrenal cortex primarily in the zona fasciculata and reticularis. ACTH-dependent Cushing syndrome is frequently associated with an ACTH-producing pituitary adenoma (Cushing disease). The left and right glands combined may weigh up to 24 g in markedly severe cases. The hyperplasia is usually diffuse, but a mixed picture of diffuse and nodular hyperplasia may be present. The outer zona glomerulosa is not usually affected by ACTH-dependent hyperplasia. Microscopic examination of the hyperplastic adrenal cortex shows lipid depletion in the zona fasciculata and reticularis. In adults the zona glomerulosa is usually quite compressed and may not be visible, while in pediatric patients this zone may be slightly hyperplastic [10].

Hyperplasia and Paraneoplastic Syndrome

Adrenocortical hyperplasia secondary to paraneoplastic or ectopic hormone production may result from neuroendocrine tumors in multiple sites including the lungs, pancreas, and thymus [11]. Other tumors such as medullary thyroid carcinomas and pheochromocytomas may also be associated with ectopic ACTH and/or CRH production [7]. The adrenals are usually larger on average than in patients with Cushing disease and the combined weight of both adrenals may be up to 30 g. The cortex is diffusely hyperplastic and tan brown. Microscopic examination shows diffuse hyperplasia of the zona fasciculata and the cells appear lipid depleted [7].

Hyperaldosteronism Associated Hyperplasia

Adrenocortical hyperplasia may be associated with hyperaldosteronism. Although adenomas are more commonly associated with hyperaldosteronism, about a third of cases may present with hyperplastic zona glomerulosa cells only or they may be a mixture of adenomas and hyperplasia [12, 13]. The glands may be of variable weight from slight enlargement to a single gland weighing 10 g or more. Microscopic examination

shows proliferation of zona glomerulosa cells and micronodules but zona fasciculata cells may also be present.

Adrenal Macronodular Hyperplasia

This form of hyperplasia is usually bilateral although the glands are often asymmetrically enlarged. The combined weight of the glands may be up to 200 g and the individual nodules may range from less than 1 cm to up to 4 cm in diameter [14–17]. This condition is ACTH independent. Microscopically the nodules may be made up of compact cells, clear cells, or mixture of these [7] (Fig. 2.1a). Interestingly, the cortical tissue between the nodules is atrophic which

indicates that the nodules are functional [16]. Massive macronodular adrenocortical disease has been analyzed at the molecular level in a recent study [18]. There were mutations of the armadillo repeat-containing 5 gene (AMRC5) [18].

Pediatric Adrenocortical Disorders Associated with Masses

Adrenal Cytomegaly and Beckwith–Wiedemann Syndrome (BWS)

This syndrome is characterized by hemihypertrophy, macroglossia, abdominal wall defects, and pancreatic islet cell hyperplasia. The adrenal is

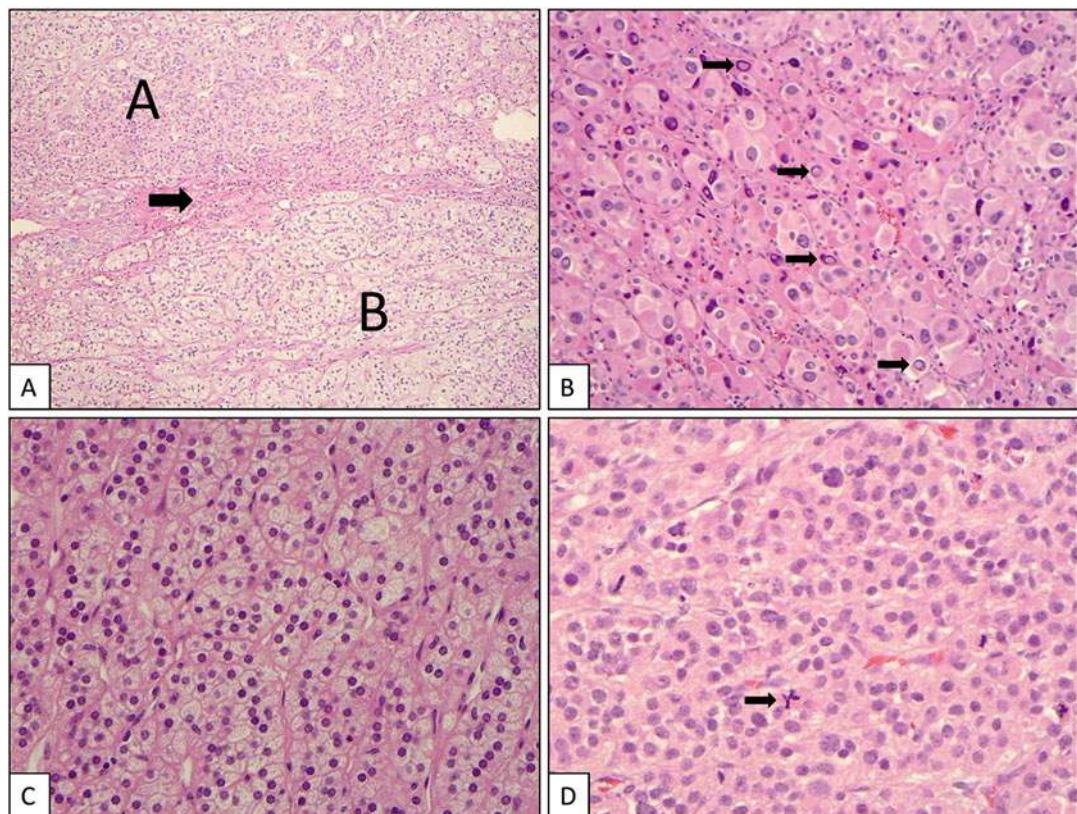


Fig. 2.1 (a) Adrenocortical macronodular hyperplasia. Two macronodules are present consisting of fasciculate-like clear cells (letters A and B). (a, b) There is atrophy of the adrenal cortex between the two nodules (arrow) indicating that the nodules are functional and secreting glucocorticoids. (b) Adrenal cortical adenoma from a patient with Beckwith–Wiedemann syndrome. The adrenal corti-

cal cells show marked cytomegaly and there is prominent cytoplasmic invagination into nuclei (arrows). (c) Histological examination of an adrenocortical adenoma shows cells with abundant clear cytoplasm and round nuclei from the zona fasciculata. (d) Adrenocortical carcinoma showing relatively uniform cells with numerous mitotic figures including atypical mitoses (arrow)

characterized by collections of enlarged cortical cells with hyperchromatic nuclei in the fetal cortex. Some cells may show intranuclear pseudoinclusions [19, 20]. Although cytomegalic cells are present in the normal fetal cortex, they are more prominent in patients with BWS. These patients have an increased risk of developing adrenocortical adenomas (Fig. 2.1b), carcinomas, neuroblastomas, Wilms' tumors along with hepatoblastomas and pheochromocytomas. The molecular pathogenesis involves dysregulated gene expression of imprinted genes within the chromosome 11p15 region [21].

Congenital Adrenal Hyperplasia

These disorders result from autosomal recessive enzymatic defects needed in the biosynthesis of adrenocortical steroids. Defects in the activity of various P450 enzymes lead to the syndrome [22, 23]. The most common defect is 21-hydroxylase deficiency (P450 c₂₁). There is inadequate production of glucocorticoids, which leads to adrenocortical stimulation by ACTH secreted from the anterior pituitary. The adrenals are enlarged with a tan-brown color and a cerebriform configuration [7]. Microscopic examination typically shows cortical cells which are lipid depleted. A variant of this disorder, lipoid congenital adrenal hyperplasia, due to mutations of acute regulatory protein, consists of pale yellow adrenals with vacuolated cells and cholesterol clefts and giant cell reaction on microscopic examination [24]. Patients may develop adrenocortical adenomas and carcinomas secondary to congenital hyperplasia [25]. They may also develop testicular tumors [26]. Both adrenal and testicular tumors are dependent on the presence of high serum levels of ACTH and are thus hormone-dependent tumors.

Adrenocortical Neoplasms

Adrenocortical Adenomas

Adrenocortical adenomas are benign neoplasms that may produce hormones associated with any of the hormones produced by the different zones of

the adrenal cortex or they may be nonfunctioning. Adrenocortical adenomas are seen at autopsy in a small percentage of cases (around 5%) [27]. Adenomas may be functional or nonfunctional. Many of the incidentalomas discovered in patients by imaging studies represent nonfunctional adenomas. The most common functioning tumors are associated with aldosterone production while adenomas with glucocorticoid production constitute the second most common group [7, 9].

Hyperaldosteronism

Adenomas associated with hyperaldosteronism (Conn Syndrome) are usually small tumors, measuring less than 2 cm in diameter, and they are commonly unilateral. They are often bright yellow on gross examination and may have a thin pseudocapsule separating them from the adjacent cortex. Microscopic examination shows cells with features of zona glomerulosa, fasciculata or reticularis, or a combination of two zones, often referred to as hybrid cells. A characteristic feature seen in many patients treated with spironolactone before surgery is the spironolactone bodies, which are lamellated eosinophilic bodies in the cytoplasm of tumor cells. A distinct halo is often present around these bodies that make them relatively easy to recognize. The tumor cells are usually small with vesicular nuclei and inconspicuous nucleoli, but some adenomas may have cells showing "endocrine atypia" which consist of larger cells and pleomorphic nuclei. Ultrastructural examination usually shows cells with prominent tubular or vesicular mitochondrial cristae and abundant smooth endoplasmic reticulum.

Cushing Syndrome

Benign tumors associated with excess glucocorticoid production and causing Cushing syndrome are usually unilateral discrete masses and can weigh up to 60 g [7, 28]. The size may be quite variable, but they are usually less than 4 cm in diameter. Larger tumors have an increased likelihood of being malignant. On cut section adenomas usually range from yellow to brown without necrosis. Cystic changes may be present especially with

larger tumors. Microscopic examination shows a discrete pseudocapsule with tumor cells arranged in nest and cords. The individual tumor cells resemble cells from the zona fasciculata (Fig. 2.1c). The nuclei are usually round and the cytoplasm contains lipid, giving the appearance of foamy cytoplasmic inclusions. Mitotic figures are uncommon in adenomas. Ultrastructural examination shows cells with abundant smooth endoplasmic reticulum, lipid droplets, and mitochondria with tubulovesicular or vesicular cristae [28]. Clonal analyses have shown that some adenomas are clonal while others may be polyclonal [29].

Adenomas Associated with Adrenogenital Syndromes

Some adenomas may be associated with virilization or feminization. It is important to rule out the possibility of a carcinoma in adrenocortical tumors associated with excessive sex steroid production [9]. Virilizing adenomas usually appear different from adenomas in patients with Cushing syndrome. The tumors are red to brown rather than yellow. Microscopically the tumors have granular eosinophilic cytoplasm without necrosis or significant mitotic figures. Ultrastructural examination shows abundant smooth endoplasmic reticulum and mitochondria with tubular-lamellar cristae.

Nonfunctional Adrenocortical Adenomas

Adrenocortical nodules are not uncommon in surgically resected adrenal glands and can be seen in about 25 % of autopsy specimens [7, 9]. Incidental nodules are also commonly detected with radiographic studies and nonfunctioning nodules are often designated as incidentalomas [30]. Multicentric nodules are often very small nodules, 2–3 cm in diameter and represent the typical nonfunctioning adenomas. They may range from bright yellow to brown with a pseudocapsule. Microscopically they are composed of cells with uniform round nuclei. The cells are reminiscent of fasciculate-type cells of the normal adrenal. On microscopic examination the adjacent cortex does not show atrophic changes as they do with functional adenomas.

Adrenocortical Carcinomas

Adrenocortical carcinomas are uncommon tumors. The incidence is around 1 per million population per year [27]. There is a bimodal distribution of carcinomas: the first occurs in the first two decades of life, and a larger peak is seen in the fifth decade of life [27, 31]. Carcinomas are slightly more common in women than in men [27]. The tumors may be associated with certain familial conditions such as Li–Fraumeni syndrome [32], Beckwith–Wiedemann syndrome [7, 9], and Lynch syndrome [33]. Most adrenocortical carcinomas are functional with estimates as high as 80 % of cases. Adrenocortical carcinomas generally weigh more than 100 g in adults with many tumors weighing more than 750 g [7, 9]. Adrenocortical carcinomas may occasionally be as small as 20–30 g. Carcinomas usually show a nodular appearance and vary in color from bright yellow to pink or brown depending on their lipid content. Areas of necrosis and hemorrhage are not uncommon and some tumors may also show focal areas of calcification. Microscopic examination shows variable patterns of growth ranging from solid to alveolar, and there is often a mixture of various patterns. Microscopic foci of necrosis are common especially in larger tumors. Unusual growth patterns such as a pseudoglandular and spindle shape appearance may be present [7, 9]. The cytoplasm of tumor cells may vary from eosinophilic to vacuolated depending on the lipid content of the cells. Some adrenocortical carcinomas are composed of cells with uniform nuclei, while other tumors may show marked nuclear pleomorphism. Mitotic activity is common including atypical mitoses (Fig. 2.1d). Some carcinomas may show prominent cytoplasmic invagination into the nuclei, but this feature does not have any diagnostic significance.

Immunohistochemical characterization of adrenocortical carcinomas is very important especially in small biopsy specimens [7, 9]. Adrenocortical carcinomas are often positive for keratin especially when very sensitive antigen retrieval techniques are used during the immunostaining procedure. Surprisingly, these tumors are also positive for synaptophysin; a common neuroendocrine

marker, but chromogranin A is consistently negative which helps in the differential diagnosis of pheochromocytomas versus adrenal cortical tumors. Adrenocortical carcinomas are usually positive for inhibin A and for MART1/Melan A. The monoclonal antibody D11 [34] is a good marker for adrenocortical tumors and helps to distinguish them from adrenal medullary tumors. The lymphatic marker D2-40 is usually positive in normal and neoplastic adrenocortical cells while adrenal medullary cells and tumors are negative for D2-40 [7]. The transcription factor steroidogenic factor 1 (SF-1) is another useful marker for adrenal cortical tumors and is relatively specific, since only a few other tissues including some anterior pituitary cells such as follicle stimulating hormone producing cells are also positive for this transcription factor [35]. Ultrastructural examination was historically important in the diagnosis of adrenocortical carcinomas, but with the advent of many relatively specific immunohistochemical stains this approach is no longer used extensively. Ultrastructural features that are helpful in the diagnosis include abundant smooth endoplasmic reticulum characteristic of steroid producing cells and mitochondria with tubular cristae.

Criteria for Malignancy in Adrenocortical Carcinomas

Distinguishing adrenocortical adenomas from carcinomas can be very difficult especially in small biopsy specimens. The studies of Weiss led to the development of histological criteria to separate adenomas from carcinomas of the adrenal cortex [36]. The criteria included necrosis, diffuse architecture, capsular invasion, atypical mitoses, sinusoidal invasion, venous invasion, and mitotic activity per 50 high power fields. Tumors with fewer than two of these criteria never metastasized, while those with more than four almost always recurred or metastasized [36]. Other workers attempted to simplify the Weiss criteria [37] by reducing the number of features needed to make a diagnosis of malignancy. Hough and coworkers had previously developed criteria for the diagnosis of adrenocortical carcinomas [38], but these have not been as robust as

the Weiss criteria. Volante and colleagues [39] developed a different set of criteria which included reticulin histochemical staining that was disrupted in carcinomas, but not in adenomas, to separate adrenocortical adenomas from carcinomas. Recent studies have shown that the use of a proliferative index measured by Ki-67/MIB1 can be another useful tool that can assist in separating adenomas from carcinomas [40].

Variants of Adrenocortical Carcinomas

Oncocytic Adrenocortical Carcinomas

Oncocytic adrenocortical carcinoma is a variant of adrenocortical carcinoma that is different enough from usual adrenocortical carcinomas that different criteria for malignancy have been proposed [41]. These tumors are characterized by the presence of abundant cytoplasmic mitochondria. There are major and minor criteria for the diagnosis of oncocytic carcinomas. The major criteria include a high mitotic rate with atypical mitoses and venous invasion. Minor criteria included necrosis, capsular invasion and sinusoidal invasion, and large tumor size. One major criterion was sufficient for the diagnosis of carcinoma, while one to four minor criteria were enough for a diagnosis of tumors of uncertain malignant potential.

Myxoid Variant of Adrenocortical Carcinoma

This variant is characterized by tumors with abundant extracellular myxoid stroma. One study suggested that these tumors have a more aggressive biological behavior compared to conventional adrenocortical carcinoma [42]. In another study, adrenocortical tumors with myxoid stroma usually behaved like carcinomas [43].

Adrenocortical Carcinomas in Pediatric Patients

The criteria used for the diagnosis of adrenocortical carcinomas in adults have not been directly applicable to tumors in the pediatric population. A study from the Armed Forces Institute of

Pathology refined the criteria for diagnosis of pediatric adrenocortical carcinomas [44]. Features that were associated with malignancy in pediatric adrenocortical carcinomas included tumors weighing more than 400 g, tumor size greater than 10.5 cm in diameter, vena cava invasion, capsular and/or vascular invasion, mitotic count greater than 15 per 20 high power fields, presence of atypical mitoses, and confluent necrosis. In multivariate analyses, vena cava invasion, necrosis, and mitotic activity independently predicted malignant behavior [44]. More recently, another group of investigators divided pediatric adrenocortical tumors into low-risk tumors which were confined to the adrenal and weighed less than 200 g, a high-risk group weighing more than 400 g and invading adjacent organs such as kidney and liver and an intermediate risk group weighing between 200 and 400 g confined to the adrenal or weighing less than 400 g with microscopic evidence of invasion into the adjacent soft tissues, and completely resected without evidence of metastatic spread [45].

Molecular Pathology of Adrenocortical Carcinomas

Molecular studies have been used to try to separate adrenocortical adenomas from carcinomas [46–48]. Gene products upregulated in carcinomas include the insulin-like growth factor (IGF) family especially IGF2 and ubiquitin-specific protease 4 (USP4) and ubiquitin degradation1-like (UFD1L). Various genes products are also down-regulated including chemokine ligand 10, retinoic acid receptor responder 2, and aldehyde dehydrogenase family member A1. Some of these gene products may have potential diagnostic importance in separating adenomas from carcinomas. Analysis of microRNAs expressed in adrenocortical adenomas and carcinomas has shown miR483-3p in many but not all carcinomas and this microRNA was overexpressed in only a small percentage of adenomas [49]. Analysis of 37 pediatric adrenocortical carcinomas by whole genome, whole exome, and/or transcriptome sequencing showed that *IGF2* was

overexpressed in 100 % of cases and that a dismal outcome was predicted with concomitant *TP53* and *ATRX* mutations [50]. Recent genome-wide analysis of genomic changes in adrenocortical adenomas and carcinomas found more alterations in carcinomas compared to adenomas and identified several novel molecular pathways associated with deregulated genes including oncostatin m. Oncostatin m signaling was identified as a potential target for treatment of patients with disseminated adrenocortical carcinomas [51]. Other molecular studies of adrenocortical carcinomas have reported recurrent alterations in genes not previously reported in adrenocortical carcinomas including *ZNRF3*, *DAXX*, *TERT*, and *MED12* [52].

Miscellaneous Adrenal Mass Lesions

Other lesions in the adrenal that should be in the differential diagnosis of mass lesions include adrenal cyst and pseudocysts, adrenal myelolipoma, and metastatic tumors to the adrenal gland [7, 9]. Adrenal cysts and pseudocyst are usually discovered incidentally during CT and MRI studies. Adrenal cysts include epithelial cysts, endothelial or vascular cysts, parasitic cysts, and pseudocysts [53]. Pseudocysts are most common and may vary from a few millimeters up to 10 cm or more in diameter. Pseudocysts do not have a true lining and usually consist of fibrin and hemorrhagic material with a fibrous wall that is sometimes calcified. Adrenal cysts and pseudocysts are almost always nonneoplastic. However, adrenal cortical adenomas, pheochromocytomas, and adrenocortical carcinomas may undergo cystic degeneration, so the wall of cysts should be carefully sampled and examined microscopically to rule out degenerative changes in a cystic neoplasm.

Adrenal myelolipoma is a benign neoplasm of the adrenal gland that is a nonencapsulated mass composed of varying amounts of mature adipose tissue and hematopoietic elements including red and white blood cell precursors and megakaryocytes [54, 55].

Metastatic tumors to the adrenal glands are relatively common and may be present in up to a third of patients with metastatic malignancies [7, 56].

Lung carcinomas are the most common primary site [7, 56]. Other frequent primary sites include breast, skin, kidney, and gastrointestinal tract. Immunohistochemical stains are very useful in determining the primary site of the tumors if the origin is not apparent clinically.

Adrenal Medullary Masses

Adrenal Medullary Hyperplasia

Adrenal medullary hyperplasia may simulate an adrenal mass in some cases [57–59]. Medullary hyperplasia is usually associated with multiple endocrine neoplasia (MEN)2A and MEN2B [57–59]. Medullary hyperplasia in von Hippel Lindau (VHL) disease and neurofibromatosis, both of which are associated with bilateral pheochromocytomas, is somewhat controversial [60, 61]. Occasional patients may have medullary hyperplasia without a family history. The hyperplasia in MEN2A and MEN 2B is usually diffuse and nodular. Morphometric studies may be needed to document a diagnosis of medullary hyperplasia in mild cases. Medullary hyperplasia may be suspected when medullary tissue is present in both alar regions of the adrenal and medullary tissue is also present in the tail of the adrenal [7]. Microscopically the medullary cells are composed of enlarged polygonal cells with abundant granular cytoplasm and round nuclei. Immunostaining with chromogranin A may assist in outlining the extent of the medullary tissue extension in the adrenal glands. Recent molecular studies have shown that in patients with MEN2A the nodular hyperplasia is clonal, suggesting that the nodules represent true neoplasm rather than simply hyperplastic nodules [7].

Pheochromocytomas

Pheochromocytomas are also referred to as intra-adrenal paragangliomas. Most pheochromocytomas arise in the adrenal glands. Tumors with similar histological features outside of the adrenal glands have been designated as pheochromocytomas by some investigators, but they should be termed as

paragangliomas or extra-adrenal paragangliomas. Pheochromocytomas vary in color from gray to pink. After exposure of the cut section to air, the surface may appear brown due to oxidation of catecholamines or adrenochromes in the tumor. Tumors may vary from around 1 to 5 cm or more. Malignant tumors are generally larger than benign pheochromocytomas. Areas of degeneration and fibrosis may be present in larger pheochromocytomas; however, these degenerative foci should not be misinterpreted as a sign of malignancy. Almost all sporadic pheochromocytomas are unilateral while familial tumors are generally bilateral and some of these are associated with medullary hyperplasia. Microscopically the tumors consist of large polygonal cells with granular cytoplasm (Fig. 2.2a). The tumors are usually basophilic, but the color varies with the fixation. Pheochromocytomas may rarely show oncocytic or clear cell features. The clear cell variant may be confused with adrenal cortical neoplasm, so immunohistochemical stains should be performed especially with small biopsies.

Other histological variants may include small cell and spindle cell variant of pheochromocytoma. A distinct feature of pheochromocytomas is the presence of eosinophilic globules that may be in the cytoplasm or in between adjacent tumor cells. The globules are thought to be derived from the membrane of secretory granules [7, 9]. These globules are not unique to pheochromocytomas, since they can also be seen in normal adrenal medulla and even in adrenocortical carcinomas. The sustentacular cells located at the periphery of nests of pheochromocytomas when the pheochromocytes form a zellballen pattern or cell nest is another component of pheochromocytomas. The function of the sustentacular cell is unknown, but they have been reported to be decreased in malignant pheochromocytomas. Sustentacular cells can be readily recognized by their spindle shape at the periphery of the cell nests or by immunostaining for S100 protein. The nuclei of pheochromocytoma cells are round to ovoid and they may show cytoplasmic invagination from the cytoplasm into the nucleus in some cells. Amyloid has been reported in varying percentages of pheochromocytomas and in some series may be as high as in 70 % of cases [62].

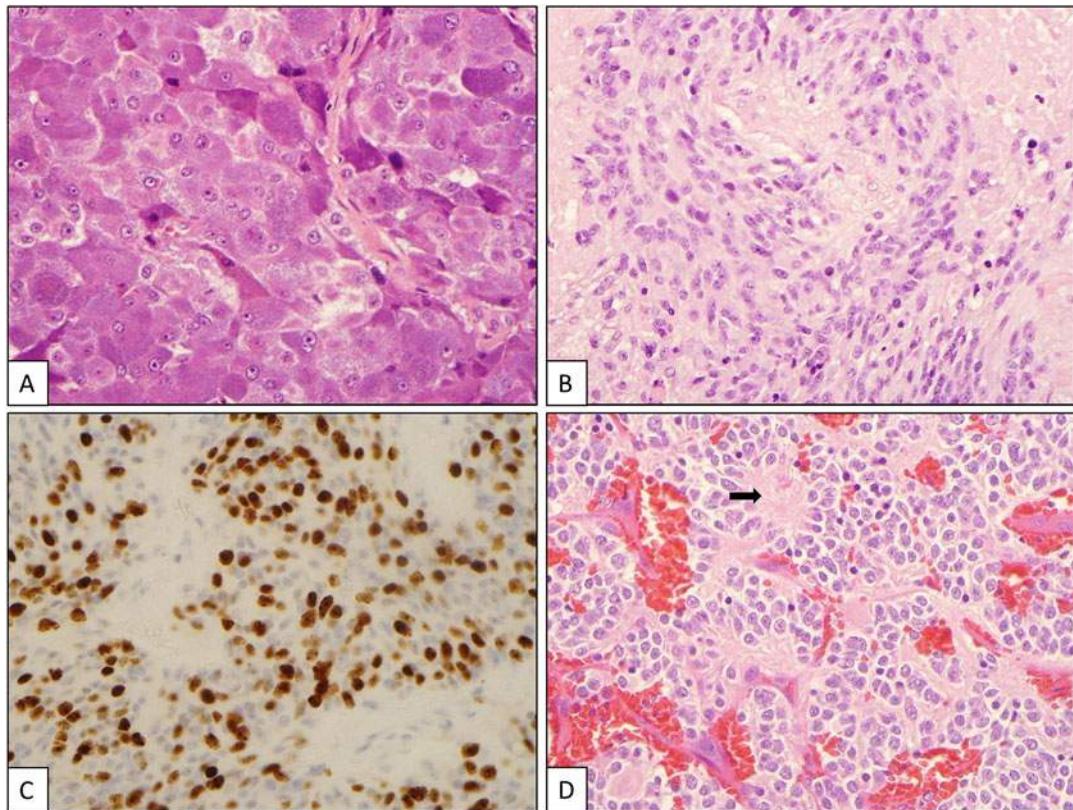


Fig. 2.2 (a) Pheochromocytoma with large polygonal cells with granular basophilic cytoplasm and round nuclei. (b) Malignant pheochromocytoma with proven liver metastasis. The tumor cells are predominantly spindle shaped and there is extensive background necrosis. (c) Immunostaining for Ki-67 in the same pheochromocyt-

toma shows a high proliferative rate (42 %) as indicated by brown nuclear staining. (d) Neuroblastoma, poorly differentiated, with prominent neuropil and Homer Wright pseudorosettes (arrow). Many congested capillaries with red blood cells are present in the background

Immunohistochemical staining is usually helpful in the diagnosis of pheochromocytomas. The tumors are usually positive for chromogranin A, Chromogranin B, and secretogranins. Although pheochromocytomas are also positive for synaptophysin, this marker is less specific since adrenal cortical tumors are often positive for synaptophysin. Antibodies directed against catecholamine synthesizing enzymes such as tyrosine hydroxylase, dopamine beta hydroxylase, and phenylethanolamine, and N-methyltransferase may help in the diagnosis of pheochromocytoma [7]. Pheochromocytomas are usually negative for keratins, although focal positivity may be present. Vimentin and neurofilament are usually positive in pheochromocytomas

as is OCT3/4, which is a marker for some germ cell tumors and stem cells [63]. GATA 3 is variably expressed in pheochromocytomas [64]. Regulatory peptides may be detected in pheochromocytomas and may lead to paraneoplastic syndrome. The more common peptides include ACTH, CRH, somatostatin, and calcitonin [7, 9]. Ultrastructural studies of pheochromocytomas show the presence of dense core secretory granules ranging in size from 200 to 800 nm in diameter. Norepinephrine- and epinephrine-containing secretory granules can be distinguished by their morphological appearance. Norepinephrine-containing secretory granules usually have a halo around the secretory granule contents of the membrane [7]. The smooth endoplasmic

reticulum is less developed in pheochromocytomas compared to steroid-producing adrenocortical tumors.

Composite Pheochromocytoma

Composite pheochromocytomas are rare variants of pheochromocytomas. They usually consist of pheochromocytomas and ganglioneuromatous elements but may also be composed of neuroblastic, ganglioneuroblastic, or malignant nerve sheath elements in addition to pheochromocytes. The pheochromocytes usually comprise the majority of the neoplasm. Composite pheochromocytomas with ganglioneuromas are usually benign neoplasms [7, 9]. They consist of pheochromocytes with neuronal or ganglion cells features along with a loose fibrillary matrix resembling neurophil. Transition between the different elements may be gradual or abrupt. The ganglionic cells may contain granular basophilic material corresponding to Nissl substance. The ganglionic cells are characterized by light pink eosinophilic cytoplasm with distinct borders and rounded eccentric nuclei and prominent nucleoli. Prominent Schwann cells are often present. Many of the reported composite pheochromocytomas have been functionally active with secretion of catecholamines. They may be associated with vasoactive intestinal polypeptide secretion leading to watery diarrhea [7, 9].

Paragangliomas

Paragangliomas are generally classified as parasympathetic or sympathetic tumors. The parasympathetic paragangliomas include tumors from the carotid body, jugulotympanic, vagal, laryngeal, aortopulmonary, and miscellaneous tumors in the head and neck region [7, 9, 65]. The tumors are usually firm and solid with a compressed pseudocapsule. On cross section they have a light brown to tan appearance and may have intersecting bands of fibrous tissues. Microscopically it is not possible to determine the anatomic location of different tumors from

their histological appearance. An organoid appearance is typical and the cytoplasm is often eosinophilic. Nuclear pleomorphism and hyperchromasia may be prominent and are not reliable criteria for evaluating malignancy. Necrosis is usually not a consistent feature in most paragangliomas. If abundant necrosis is present, one should consider the likely possibility that the tumors were embolized before surgery. The sustentacular cells are similar to those in pheochromocytomas and are positive for S100 protein. The chief cells are positive for chromogranin and synaptophysin.

Sympathoadrenal paragangliomas arise predominantly in the retroperitoneum from the upper abdomen to the pelvic floor. The anatomic region corresponding to the organs of Zuckerkandl at the bifurcation of the aorta has the highest volume of paraganglionic tissue outside of the adrenal medulla. Other anatomic sites of these paragangliomas include the urinary bladder, gall bladder, spermatic cord, prostate glands, pancreas, uterus, and renal hilum. The gross appearance of the tumors is well circumscribed and may appear encapsulated [9]. They may range from a few centimeters in diameter such as in the urinary bladder to 8 cm or larger. Functional tumors are generally smaller than the nonfunctional ones. Microscopically the tumors consist of anastomosing cords of cells or trabecular arrangement with acidophilic granular cytoplasm. Nuclear pleomorphism may be prominent. Nuclear pseudo-inclusions are more common than in head and neck paragangliomas. Ganglion-like cells may be present in some tumors. Rarely, paragangliomas and pheochromocytomas may have a black appearance due to the presence of melanosomes and premelanosomes on ultrastructural examination.

Multicentric and Familial Paragangliomas

Familial paraganglioma syndromes are associated with the succinate dehydrogenase gene family mutations including *PGL1* (*SDHD*), *PGL2* (*SDHAF2*), *SDHC*, and *PGL4* (*SDHB*). *PGL 1, 2, and 3* are associated with paragangliomas of the head and neck region. *PGL1* is the most common paraganglioma syndrome and is associated with a

low incidence of malignancy while *PGL4* with mutations of *SDHB* has the greatest association with malignancy [66].

Malignant Pheochromocytoma/ Paragangliomas

Diagnosis of malignancy in pheochromocytomas/paragangliomas is very difficult, since there are no absolute criteria to predict the behavior of these neoplasms. Earlier studies suggested that features more commonly associated with malignancy included larger tumor size with a mean weight of 383 g for malignant tumors compared to 73 g for benign tumors, vascular invasion, confluent tumor necrosis, and extensive local invasion [7, 9, 67]. Other features that were less specific in distinguishing benign from malignant tumors included decrease number of hyaline globules in malignant pheochromocytomas and decrease numbers of sustentacular cells in malignant tumors [7, 9]. However, there was usually a great deal of overlap of these features in benign and malignant pheochromocytomas/paragangliomas.

Other approaches have been used to try to separate benign and malignant pheochromocytomas/paragangliomas. The use of cytomorphometry was attempted for some time, since benign tumors had a mode corresponding to diploid population of DNA content and a wide range of values with nuclei up to 40n. In contrast, malignant pheochromocytomas were hyperdiploid or triploid with a smaller range of values [7]. Cytomorphometric analysis is not currently widely used in separating benign and malignant pheochromocytomas. The proliferating marker Ki-67/MIB-1 has been used as an adjuvant marker in separating benign and malignant pheochromocytomas (Fig. 2.2b, c). Because the proliferation rate of pheochromocytomas is relatively low, a cut point of 3% has been used in separating the two groups [7]. A recent study has used Ki-67 as one of several parameters in separating benign and malignant neoplasm as will be discussed later.

Pheochromocytoma of the Adrenal Gland Scaled Score (PASS)

Thompson [68] proposed the pheochromocytoma of the adrenal gland scaled score (PASS) system to separate benign from malignant pheochromocytomas based on a clinicopathologic and immunophenotypic study of 100 cases. Fifty histologically malignant and 50 histologically benign pheochromocytomas of the adrenal gland were studied. Histologically, the cases of malignant pheochromocytomas of the adrenal gland demonstrated larger size, invasion such as vascular, capsular, and periadrenal adipose tissue spread, large nests or diffuse growth, and focal or confluent necrosis, high cellularity, tumor cell spindling, cellular monotony, increased mitotic figures, atypical mitotic figures, profound nuclear pleomorphism, and hyperchromasia more frequently than benign tumors. The PASS system weighted for these specific histologic features could be used to separate tumors with a potential for a biologically aggressive behavior (PASS greater or equal to 4) from tumors that behave in a benign fashion (PASS <4). The pathologic features that are incorporated into the PASS correctly identified tumors with a more aggressive biologic behavior [68].

PASS was one of the earlier scoring systems for the diagnosis of adrenal pheochromocytomas. However, the reproducibility and clinical significance of the PASS system has been controversial [69, 70]. One study [69] found that a higher threshold of 6 was indicative of malignant behavior but recommended that patients with a PASS score 4 should be closely followed. Another group of pathologists [70] examined the utility of PASS by reviewing an independent single institutional cohort of adrenal pheochromocytomas as evaluated by 5 multi-institutional pathologists with at least 10-year experience in endocrine pathology. Significant interobserver and intraobserver variability in the PASS score with variable interpretation of the underlying components was reported, suggesting that this was not a very reliable approach even for expert endocrine pathologist [70].

Grading System for Adrenal Pheochromocytomas and Paragangliomas (GAPP System)

The Pheochromocytoma Study Group in Japan analyzed 163 tumors including 40 metastatic pheochromocytomas and paragangliomas using their grading system for adrenal pheochromocytoma and paraganglioma (GAPP) System [71]. The tumors were scored based on GAPP criteria as follows: histologic pattern, cellularity, comedo-type necrosis, capsular/vascular invasion, Ki67 labeling index, and catecholamine type. All tumors were scored from 0 to 10 points and were graded as one of three types: well differentiated, moderately differentiated, and poorly differentiated. GAPP scores of the nonmetastatic and metastatic groups were 2.08 ± 0.17 and 5.33 ± 0.43 , (mean \pm SE, $P < 0.001$), respectively. The mean number of years until metastasis after the initial operation was 5.5 ± 2.6 years. The 5-year survival of these groups was 100, 66.8, and 22.4 %, respectively. In addition, negative immunoreactivity for succinate dehydrogenase gene subunit B (*SDHB*) was observed in 13 (8 %) moderately or poorly differentiated tumors, and 10 of the 13 (77 %) had metastases [71].

Molecular Alterations in Pheochromocytomas/Paragangliomas

Familial pheochromocytomas are associated with MEN2A, MEN2B, VHL disease, and neurofibromatosis type I (NF1) [7, 9, 71]. In MEN2A and 2B disease there is a germline activating mutation in the *RET* protooncogene. In VHL-associated pheochromocytomas, the *VHL* gene usually contains a mutation at codon 238 [72]. Patients with NF-associated pheochromocytomas usually have mutation of the *NF1* gene [73]. Recent studies of pheochromocytomas/paragangliomas have reported germline or somatic mutations in *THEM127*, *H-RAS*, *KIF1B*, *HIF2* alpha, *PHD2*, and fumarate hydratase (*FH*) genes in addition to the *RET*, *VHL*, and *NF1* genes [66].

Mutations in the succinate dehydrogenase (SDH) mitochondrial complex II, an enzyme

complex that catalyzes the oxidation of succinate to fumarate in the Krebs cycle and participates in the electron transport chain, are present in some pheochromocytomas as well as in paragangliomas. *SDHx* genes are composed of four subunits encoded by the corresponding genes: *SDHA*, *SDHB*, *SDHC*, and *SDHD*. Complex subunits A and B constitute the catalytic core of the enzyme, while subunits C and D anchor the complex to the inner mitochondrial membrane. In general, inactivating mutations in one of the *SDHx* genes lead to accumulation of succinate and formation of reactive oxygen species, stabilizing HIF1 protein and activating hypoxia-dependent pathways [74, 75]. The few individuals with *SDHA* mutations described so far have presented with distinct phenotypic characteristics of pheochromocytomas/paragangliomas including sympathetic (abdominal and thoracic), and parasympathetic head and neck paragangliomas. Both missense and nonsense mutations have been reported without any genotype–phenotype correlations [74–78].

SDHB mutations have been reported in some intra-adrenal tumors, but mostly in extra-adrenal sites. Recurrence and malignancy were strongly associated with *SDHB* mutations and suggested that the presence of *SDHB* mutants should be considered a high-risk factor for malignancy or recurrence [77–80]. *SDHD* mutation is associated with head and neck paragangliomas. As with other familial paragangliomas, these patients are more likely to have multifocal disease. Both *SDHB* and *SDHD* mutated gene-related pheochromocytomas and paragangliomas typically secrete norepinephrine and dopamine, or dopamine alone. *SDHC* mutations were initially described in head and neck paragangliomas, but have since been reported in adrenal pheochromocytomas and paragangliomas at other sites [80, 81].

Several investigators have reported the use of immunohistochemical methods with antibodies to the *SDHx* proteins to screen for mutations of *SDHB*, *SDHC*, and *SDHD* genes in familial disease associated with the pheochromocytoma-paraganglioma syndrome [82, 83]. Loss of *SDHx* expression is suggestive of a mutation with these antibodies. An internal control such as endothelial

cells should show positive staining for SDHx to support the immunohistochemical method. These screening methods should be validated with more conventional molecular screening to detect the specific mutation.

MicroRNA profiling has been used to try to separate benign and malignant pheochromocytomas [84, 85]. MiR-483-5p was reported to be overexpressed in malignant tumors while miR-15a and miR-16 were underexpressed in the malignant tumors [84]. Another study also observed overexpression of miR-483-5p as well as miR-183 and miR-101 in malignant pheochromocytomas compared to benign tumors [85]. A comprehensive genomic landscape analysis of pheochromocytoma/paraganglioma indicated that the main drivers were distinct germline and/or somatic mutations in susceptibility genes and unique gene alterations were noted [86]. There were miRNA clusters 182/196/183 which were associated with *SDHB*-mutated tumors and were associated with some malignant traits while silencing of the imprinted DLK1-MEG3 miRNA cluster, which included a long noncoding RNA, was noted in a specific subgroup of sporadic tumors [86].

Peripheral Neuroblastic Tumors

Peripheral neuroblastic tumors (pNTs) refer to a group of tumors including neuroblastoma, ganglioneuroblastoma, and ganglioneuroma, with neuroblastoma accounting for 97% of the cases. They are by definition embryonal tumors of the sympathetic nervous system arising from the neural crest [87]. They almost exclusively occur in children and are the most common solid extracranial tumor in children [88]. Due to its childhood onset, multiple maternal and prenatal factors have been investigated and suggested to be pathogenic, including prenatal exposures to tobacco, alcohol and pesticides, maternal medication or drug use, folate deficiency, gestational diabetes mellitus, small size for gestational age, congenital abnormalities, and maternal history of fetal loss. However, none of these possible associations were confirmed in large studies [87].

pNTs typically arise in the adrenal medulla, paravertebral sympathetic ganglia, and sympathetic paraganglia, with the adrenal being the most common site and accounts for 40% of the cases [88]. They are heterogeneous tumors and demonstrate many unique behaviors, including involution/spontaneous regression and maturation. Due to their prevalence in children and the aggressiveness of some cases, nation-wide screening programs based on biochemical profiling were launched in different regions. However, so far only the screening programs in Japan focusing on older children showed some potential benefits [81, 88]. Early detection of pNTs is particularly challenging. The once attractive concept of *in situ* neuroblastoma, small nodules of neuroblastic cells found within the adrenal gland of asymptomatic children, were found to be in fact remnant of normal fetal development [89]. Recent studies revealed that malignant neuroblastomas and developing neuroblasts share similar genetic profiles [90], reinforcing the difficulty of early diagnosis of pNTs at the molecular level.

Classification

International Neuroblastoma Pathology Classification divides pNTs into four categories based on the level of differentiation and the arrangement of the undifferentiated components: neuroblastoma (Schwannian stroma-poor); ganglioneuroblastoma, intermixed (Schwannian stroma-rich); ganglioneuroma (Schwannian stroma-dominant); and ganglioneuroblastoma, nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor) [91]. Grossly, pNTs demonstrate considerable variations but are usually solid, white tan, with varying amounts of hemorrhage, necrosis, cystic degeneration, and calcification. Typically, the less mature component of pNTs is grossly associated with increased hemorrhage and/or necrosis and vague borders. Neuroblastomas are the least differentiated class of pNTs and are usually hemorrhagic with vague, bulging lobules. Ganglioneuroblastomas are more homogenous than neuroblastomas, and more frequently demonstrate calcification and cystic

degeneration. Different from the intermixed type, the nodular type of ganglioneuroblastomas by definition has grossly identifiable hemorrhagic nodules with well-demarcated borders, corresponding to less differentiated area. Ganglioneuromas are the most differentiated tumors among the pNT spectrum, usually well circumscribed with resilient texture, trabecular or whorled appearance, and minimal hemorrhage.

Microscopically, the classification of pNTs reflects the presence and amount of Schwannian stroma as well as the cytological features of the tumor cells. Neuroblastomas contain none or minimum of up to 50% of Schwannian stroma. They are further divided into undifferentiated, poorly differentiated, and differentiating subtypes. Undifferentiated subtype of neuroblastomas is filled with small to medium size blue monotonous cells with minimal cytoplasm on H&E staining. The nuclei have the characteristic salt-and-pepper appearance and may contain distinct nucleoli. Identifiable background thin neuritic process (neuropil) is absent. Tumors of the poorly differentiated subtype contain mainly undifferentiated cells (Fig. 2.2d). They are separated from the undifferentiated subtype by the presence of neuropil and less than 5% of differentiating tumor cells that have appreciable nuclear enlargement, eosinophilic cytoplasm, and clearer cell borders. Tumors of the differentiating subtype are further matured compared to poorly differentiated subtype, containing more than 5% of differentiating tumor cells. Ganglioneuroblastomas by definition contain more than 50% of Schwannian stroma. The tumor population consists of a mixture of more than 5% of the differentiating and undifferentiated tumor cells, with the latter forming either microscopically (intermixed) or macroscopically (nodular) distinct clusters. Ganglioneuromas do not contain any undifferentiated tumor cells and are with dominant Schwannian stroma. They are further divided into maturing (when still contain differentiating neuroblasts) and mature (when no longer contain any neuroblasts) subtypes [91–95].

pNTs are usually considered “enigmatic” because the standard grading and staging systems

Table 2.1 Prognosis of poorly differentiated and differentiating neuroblastoma [95]

Subtype	MKI	Age (year)	Prognosis
Poorly differentiated	>4 %	Any	UH
	Any	>1.5	UH
	<4 %	<1.5	FH
Differentiating	Any	>5	UH
	<4 %	<1.5	FH
	>4 %	Any	UH
	<2 %	1.5–5	FH
	>2 %	1.5–5	UH

MKI mitosis-karyorrhexis index, *UH* unfavorable histology, *FH* favorable histology

often fail to predict clinical behaviors, especially the occurrence of involution. It was gradually realized that in addition to the level of differentiation, patient's age, cellular turnover index (reflexed by mitosis-karyorrhexis index, MKI, defined by the number of cells with mitosis and karyorrhexis of every 5000 cells), and the presence of macroscopic nodules of neuroblasts are important factors for risk stratification. This knowledge is summarized in International Neuroblastoma Pathology Classification (Table 2.1), which was proposed by International Neuroblastoma Pathology Committee based on the Shimada classification [91] in 2001 [93, 94] and was subsequently revised in 2003 [95]. Based on histologic features and the age at diagnosis, pNTs can be divided into those with “favorable histology” (FH) and “unfavorable histology” (UH). In general, increased tumor cell differentiation, younger age (<18-month old), and low MKI (<2%) are associated with FH; while decreased tumor cell differentiation, older age (>5-year old), and high MKI (>4%) are associated with UH. It was originally noted that the presence of macroscopic nodules of neuroblasts within ganglioneuroblastoma conveys a universal unfavorable prognosis [91]. However, subsequent analysis revealed that the observation was not entirely accurate and younger patients with macroscopic nodules of poorly differentiated or differentiating rather than undifferentiated neuroblasts may still have favorable prognosis [95].

Molecular Pathology of pNTs

A familial history of pNTs is observed in about 1% of patients with pNTs and autosomal-dominant inheritance is suggested. Three genes involved are PHOX2B, ALK, and NF1, which are also important for normal development and differentiation of neural crest. Hirschsprung's disease, Congenital Central Hypoventilation Syndrome, Noonan syndrome, and Costello syndrome are known to be associated with increased risk of pNTs [96]. Single nucleotide polymorphisms (SNPs) of several additional genes were suggested to be related to the susceptibility of pNTs, including *BARD1*, *LINC00340*, *LMO1*, *DUSP12*, *DDX4/IL31RA*, *HSD17B12*, *LIN28B*, *HACE1*, *CHEK2*, *PINK1*, and *BARD1*. However, the risk that neuroblastoma may recur in families with these risk alleles is estimated to be very low [87]. The presence of recurrent somatic mutations in sporadic pNTs is interestingly low, and very few genes were suggested to separate low- and high-risk pNTs. In the most recent effort to characterize recurrent mutations in sporadic pNTs by whole genome sequencing and exome sequencing, it was found that the median exonic mutation frequency was only 0.60 per Mb, and somatic mutations in only a few genes, namely, *ALK*, *PTPN11*, *ATRX*, *MYCN*, and *NRAS*, are associated with high-risk pNTs at low frequency [97]. It is possible that the majority of high-risk pNTs are driven by rare germline variants, copy number alternations, and epigenetic modifications.

MYCN amplification is seen in about 20–30% cases of pNTs and was known for its association with unfavorable prognosis [91, 93]. It is frequently seen in pNTs with advanced stage and rapid progression. The ploidy of tumor cells also appears to affect prognosis, with hyperdiploidy being associated with favorable prognosis. Both *MYCN* amplification and ploidy are included in the current Children's Oncology Group Risk Group Classification [87, 98]. Possible prognostic chromosomal abnormalities include loss of heterozygosity of chromosome 11q, 1p, 14q and gain of 17q [87, 99, 100]. In terms of additional prognostic genetic permutations, *TRKB* (*NTRK2*) transcript is suggested to be expressed primarily

in highly aggressive *MYCN*-amplified tumors [87, 90]. Due to the routine need of analysis of *MYCN* amplification and ploidy in pNTs with snap-frozen tissues and cell culture in addition to histological examination, it is advisable to provide sufficient tissues when obtaining a biopsy [89].

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Genetics of Benign Adrenocortical Tumors

3

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Abbreviations

AC	Adenyl cyclase	GPCRs	G protein-coupled receptors
ACTH	Adrenocorticotropic hormone	Heterozygous	A genotype with two different alleles of a gene for a particular trait
AIMAH	ACTH-independent macronodular adrenal hyperplasia	Homozygous	A genotype with the same allele of a gene for a particular trait
Alleles	Alternative forms of a gene	MMAD	Massive macronodular adrenocortical disease
AMP/ATP	Adenosine monophosphate/adenosine triphosphate	Mutations	Alteration of genetic material producing a new variation
BAH	Bilateral adrenocortical hyperplasia	PBAD	Primary bimorphic adrenocortical disease
cAMP	Cyclic adenosine monophosphate	PBMAH	Primary bilateral macronodular adrenocortical hyperplasia
CNC	Carney complex	PDEs	Phosphodiesterases
CS	Cushing syndrome	Phenotype	Detectable expression of a genotype
Genes	Units of inheritance at specific locations (loci) on a chromosome	PKA	Protein kinase A
GMP/GDP/GTP	Guanosine monophosphate/guanosine diphosphate/guanosine triphosphate	PPNAD	Primary pigmented micronodular adrenocortical disease
		PRKAR1A	Protein kinase A regulatory subunit type 1

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Introduction

Benign adrenocortical tumors (ACT) represent a heterogeneous group of lesions of the adrenal cortex. Somatic and germline mutations in key molecular pathways, including cyclic AMP (cAMP) and *Wnt*-signaling pathways, have been

shown to play pivotal roles in the development of ACTs. Even in cases where there are no known mutations [1], the cAMP pathway in particular appears to be involved. The discovery of mutations in *GNAS*, which encodes the alpha subunit ($G_{s\alpha}$) of the stimulatory guanine nucleotide-binding protein, first reported in benign cortisol-producing adenomas (CPA) of patients with McCune–Albright syndrome (MAS), paved the way for identifying the cAMP-signaling pathway as the most important one in the pathogenesis of benign cortisol-producing ACTs. This was rapidly followed by the discovery of mutations in the regulatory subunit type 1- α (RI α) of protein kinase A (PKA, *PRKAR1A* gene) and phosphodiesterase-11A and -8B (*PDE11A* and *PDE8B* gene, respectively) in Carney complex (CNC) and isolated adrenal hyperplasia, and the recently identified germline mutations in the tumor suppressor gene *ARMC5* (*armadillo repeat containing 5*) and somatic mutations in *KCNJ5*, which have been implicated in the majority of primary bilateral macronodular adrenocortical hyperplasia (PBMAH) [2, 3] and aldosterone producing adenomas (APA), respectively. Other syndromes with increased predisposition to benign ACT include Carney triad (CT), Carney–Stratakis syndrome (CSS), familial adenomatous polyposis (FAP), and hereditary leiomyomatosis and renal cancer syndrome (HLRCS). In this chapter, we discuss the genetic and molecular mechanisms responsible for the formation of benign ACTs. We focus on the most recent genetic advances, diagnosis, and patient counseling in these conditions.

Classification of Benign Adrenocortical Tumors (ACT)

A comprehensive classification of ACT was proposed in 2007 (Table 3.1) [3]. In brief, ACT are grossly divided into adrenocortical adenomas (ACA), adrenocortical hyperplasia, and adrenocortical cancer (ACC) [3]. These lesions can be unilateral or bilateral. ACA are classified on the basis of their radiographic and biochemical charac-

teristics as being either functional or nonfunctional and benign or malignant. In a postmortem series, ACAs were present in 5% of cases, while adrenocortical hyperplasia in 36% [4]. Conversely, PBMAH is estimated to affect 10% and 15% of Cushing syndrome (CS) in young adulthood and childhood, respectively [3], with likely higher figures in subclinical CS. Approximately 75–90% of ACT leading to CS are due to a unilateral and benign CPA, with the remaining majority being primary pigmented micronodular adrenocortical disease (PPNAD), isolated massive adrenocortical disease (iMAD), and PBMAH [5].

PBMAH was first described in 1964 [6] and was previously called massive macronodular adrenocortical disease (MMAD), bilateral macronodular adrenal hyperplasia (BMAH), or ACTH-independent macronodular adrenocortical hyperplasia (AIMAH). Given the recent discovery of a local intra-adrenal secretion of adrenocorticotropic hormone (ACTH) with an autocrine/paracrine effect on cortisol secretion through aberrant G-protein-coupled receptors (GPCRs) [7, 8], the term PBMAH was proposed. Asynchronous involvement of only one adrenal gland in PBMAH is rare, but could pose a diagnostic challenge to clinicians. Secondary bilateral adrenocortical hyperplasia or adenomatous formation due to excess ACTH stimulation of the adrenal glands from either ectopic ACTH secretion or Cushing disease (dysregulated cortisol production from a pituitary ACTH-secreting tumor), which may become autonomously functioning, should be differentiated from primary ACT due to genetic causes as the workup and management differs.

Cortisol-producing bilateral adrenocortical hyperplasias (BAH) are divided into micronodular (<1 cm in diameter) or macronodular (>1 cm in diameter) [3]. An additional feature is pigmentation (i.e., mainly lipofuscin) within the lesion or the surrounding adrenal cortex, which characterizes a particular type of BAH, called PPNAD. Thus, a careful histologic examination of the adrenal tissue by an experienced pathologist is a critical step in subtyping the various types of BAH.

Table 3.1 Classification and characteristics of benign adrenocortical tumors

Adrenocortical lesions	Genes (locus)	Histopathology	Characteristics
ACA	<i>Nonfunctional</i> <i>CTNNB1</i> (3p22.1) <i>PRKAR1A</i> (17q22-24)	<ul style="list-style-type: none"> ACA are small (<5 cm), well circumscribed, bright yellow due to their enriched cytoplasmic lipid Nonneoplastic adrenal cortical nodules may be difficult to differentiate from ACA; they may be multifocal and bilateral APA is predominantly composed of cells similar to fasciculata; hyperplasia of the glomerulosa layer may be seen. Others may appear like fasciculata, glomerulosa, and reticularis 	<ul style="list-style-type: none"> Presence of aberrant GPCRs in CPAs Can be associated with MEN-1, FAP, MAS, HLRCS, CNC, Carney triad, and others Majority of APA harbor a <i>KCNJ5</i> mutation Germline mutations in <i>ARMC5</i> gene may be seen in APA of patients with African Americans decent <i>ATP2B3</i> has been implicated in APA of females with a more severe phenotype <i>CACNA1D</i> mutants in APA are more common in males
APA:	<i>CTNNB1</i> (3p22.1) <i>KCNJ5</i> (11q24.3) <i>ARMC5</i> (16p11.2) <i>ATP1A1</i> (1p13.1) <i>ATP2B3</i> (Xq28) <i>CACNA1D</i> (3p14.3) <i>CACNA1H</i> (16p13.3)	<ul style="list-style-type: none"> <i>CTNNB1</i> (3p22.1) is associated with a more severe phenotype <i>ATP1A1</i> (1p13.1) is associated with a more severe phenotype <i>ATP2B3</i> (Xq28) is associated with a more severe phenotype <i>CACNA1D</i> (3p14.3) is associated with a more severe phenotype <i>CACNA1H</i> (16p13.3) is associated with a more severe phenotype 	<ul style="list-style-type: none"> Germline mutations (p.M1549V) in <i>CACNA1H</i> were identified in early onset PA and may represent a new subtype of familial aldosteronism Somatic activating mutations of <i>PRKACA</i> (c.617A>G/p.L206R) with an estimated incidence of approximately 42% in CPA Somatic mutations in <i>GNAS</i> were identified in 5-17% of CPA The somatic allelic losses of <i>PRKAR1A</i> were found in 23% of CPA that were smaller tumors and exhibited a paradoxical increase in urinary cortisol levels after dexamethasone suppression, due to increased glucocorticoid receptor expression in ACT <i>CTNNB1</i> (p.S45P, p.S45F) in approximately 23.1% of CPA
CPA:	<i>PRKACA</i> (19p13.1) <i>GNAS</i> (20q13) <i>PRKAR1A</i> (17q22-24) <i>CTNNB1</i> (3p22.1)	<ul style="list-style-type: none"> CPA is composed of cells similar to fasciculata, with adjacent cortical atrophy. Heterogeneity with lipid-depleted cells admixed may be present 	<ul style="list-style-type: none"> Middle age Associated with MEN-1, FAP, MAS, HLRCS, CNC, isolated (AD) Majority of lesions with ectopic GPCRs (vasopressin, serotonin, catecholamines, luteinizing hormone) PBMAH carry the ability of intra-adrenal production of ACTH with an autocrine/paracrine effect on cortisol production FDCS is a subtype of PBMAH with aberrant GPCR to gastrointestinal inhibitory polypeptide
PBMAH	<i>ARMC5</i> (16p11.2) <i>MEN1</i> (11q13) <i>FH</i> (1q42.3-43) <i>APC</i> (5q22.2) <i>PRKAR1A</i> (17q22-24) <i>PDE11A</i> (2q31.2) <i>GNAS</i> (20q13)	<ul style="list-style-type: none"> Distinct adenomas (usually two or three), > 1 cm, with internodular atrophy or hyperplasia without atrophy 	<ul style="list-style-type: none"> Middle age Associated with MEN-1, FAP, MAS, HLRCS, CNC, isolated (AD) Majority of lesions with ectopic GPCRs (vasopressin, serotonin, catecholamines, luteinizing hormone) PBMAH carry the ability of intra-adrenal production of ACTH with an autocrine/paracrine effect on cortisol production FDCS is a subtype of PBMAH with aberrant GPCR to gastrointestinal inhibitory polypeptide

(continued)

Table 3.1 (continued)

Adrenocortical lesions	Genes (locus)	Histopathology	Characteristics
PBAd	<i>GNAS</i> (20q13)	• Distinct adenomas (>1 cm), with occasional microadenomas and internodular atrophy	• Infants and very young children • MAS
i-PPNAD	<i>PRKAR1A</i> (17q22-24) <i>PDE11A</i> (2q31.2) <i>PDE8B</i> (5q13) <i>PRKACB</i> (2p16)	• Microadenomatous (<1 cm) hyperplasia with pigmentation	• Children and young Adults • Lentiginosis in few cases • c.709-7del6 mutation and c.1A>G/p.M1V substitution in <i>PRKAR1A</i>
c-PPNAD	<i>PRKAR1A</i> (17q22-24, CNC1 locus) <i>PRKACB</i> (2p16, CNC2 locus)	• Microadenomatous (<1 cm) hyperplasia with (mostly) internodular atrophy and pigmentation	• Children, young, and middle-aged adults • Disease at a younger age and a higher frequency of myxomas, schwannomas, and thyroid and gonadal tumors than patients without <i>PRKAR1A</i> mutations • In-frame deletion of exon 3 and the c.708+1G>T mutation appears to confer a more severe CNC phenotype, while the splice variant c.709(-7-2)del6 and the initiation alternating substitution c.1A>G/p.M1Vp have been associated with incomplete penetrance of CNC, as seen in i-PPNAD • CNC1: The hot spot c.491-492delTG mutation is most closely associated with lentigines, cardiac myxoma, and thyroid tumors when opposed to all other <i>PRKAR1A</i> mutations • Expressed RIα mutant protein present with more severe and aggressive CNC phenotype • CNC2: Sporadic disease later in life with a lower frequency of myxomas, schwannomas, thyroid and LCCSCT
iMAD	<i>PDE11A</i> (2q31.2) <i>PDE8B</i> (5q13) <i>PRKAR1A</i> (2q31.2) <i>PRKACA</i> (19p13.1) 2p12-p16 5q	• Microadenomatous (<1 cm) hyperplasia with internodular hyperplasia and limited or absent pigmentation	• Mostly children and young adults • May be associated with a paradoxical rise of glucocorticoid excretion during the Liddle's test (1 mg overnight and low and high dose dexamethasone suppression tests)

ACA adrenocortical adenoma, *APC* adenomatous polyposis coli gene, *c-PPNAD* CNC-associated PPNAD, *CPA* cortisol-producing adenoma, *CNC* Carney complex, *FAP* familial adenomatous polyposis, *FDCS* food-dependent Cushing syndrome, *GNAS* gene coding for the stimulatory subunit α of the G-protein (Gαs), *GPCR* G-protein-coupled receptor, *HLRCS* hereditary leiomyomatosis and renal cancer syndrome, *i-MAD* isolated micronodular adrenocortical disease, *i-PPNAD* isolated macronodular adrenocortical hyperplasia, *PBMAD* primary bilateral macronodular adrenocortical disease, *PBAd* primary pigmented micronodular adrenocortical disease, *PRKAR1A* protein kinase, cAMP-dependent regulatory, type I, α gene

The classification of BAH is summarized in Table 3.1. Briefly, the micronodular subtypes are usually diagnosed in children and young adults, and are either pigmented (c-PPNAD, familial as seen in CNC, or isolated, i-PPNAD) or not pigmented (iMAD) [3]. The macronodular subtypes, which are usually diagnosed in adults over the age of 50, may be sporadic or familial. Syndromic forms are seen with mutations in *ARMC5*, *APC*, *MEN1*, *FH* and the CT, CSS, and HLRCS. Other subtypes of macronodular PBMAH include primary bimorphic adrenocortical disease (PBAD), as seen in MAS, and lesions with GPCRs that produce excess cortisol only in response to certain endogenous factors (e.g., gastrointestinal inhibitory polypeptide, GIP), as seen with food-dependent Cushing syndrome (FDCS). The different histopathologic characteristics of these lesions are summarized in Table 3.1.

Molecular Pathways in Benign Adrenocortical Tumors

Two major molecular pathways in adrenocortical development have been implicated in the formation of ACT and include the cAMP and *Wnt*-signaling pathways. Briefly, GPCRs (e.g., melanocortin 2 receptor, MC2R) undergo conformational changes in response to a variety of extracellular stimuli, including catecholamines, ACTH, or neurotransmitters (Fig. 3.1). In the case of glucocorticoids, ACTH activates MC2R, a seven-transmembrane receptor, which leads to activation of adenyl cyclase (AC) through G α subunit (encoded by *GNAS*) (see Fig. 3.1). This step exchanges GDP for GTP, which in turn converts ATP into cAMP, activating protein kinase A (PKA). PKA is a holoenzyme that consists of a tetramer of two homo- or heterodimers regulatory subunits (R1 α , R1 β , R2 α , and R2 β), and catalytic subunits (C α , C β , C γ , and PRKX) that are encoded by several genes [9]; their dissociation in the presence of cAMP enables phosphorylation of PKA targets, leading to gene expression to mediate cell growth, differentiation, and hormone production (e.g., cortisol). The major

function of the regulatory subunits of PKA is to keep the catalytic subunits inactive in the absence of cAMP [9].

Alterations in any of these complex steps in the cAMP-dependent signaling pathway may predispose to the formation of ACT (Fig. 3.2). The first alterations were reported in *GNAS* as seen in patients with MAS, followed by CNC through the inactivating mutations in *PRKARIA* of PKA. This leads to constitutive activation of the pathway by increasing the availability of the PKA catalytic subunits (see Fig. 3.2). However, PKA signaling may either inhibit or stimulate cell proliferation depending on its specific role in the cell cycle [10, 11]. The variability in PKA's cell growth control may explain, in part, why some biochemically active ACT are small and difficult to detect clinically.

The *Wnt*-signaling pathway consists of two major pathways: a β -catenin dependent and a β -catenin independent. Briefly, the *Wnt*/ β -catenin signaling pathway consists of binding of the ligand to a series of Frizzled family receptors, such as LRP6, which activates phosphoproteins that inhibit the phosphorylation of β -catenin (see Fig. 3.1). The nuclear accumulation of β -catenin leads to the transcription of important genes, such as *WISP2*, *CTNNB1*, and *GSK3B*, as seen in PBMAH and PPNAD [12]. Both signaling pathways share the downstream activation of certain oncogenic signals but differ substantially in their effects on others depending on the adrenocortical lesion [13]. This differential effect may explain, in part, why some somatic activating or inactivating mutations in the same signaling pathway leads to different types of ACT.

Familial Syndromes

Several familial syndromes have been described in association with benign ACTs. Familial BAH is discussed under Section “Genetic Aberrations in Adrenocortical Tumors,” while diseases associated with ACC such as Li–Fraumeni syndrome and Beckwith–Wiedemann syndrome are discussed in Chap. 4.

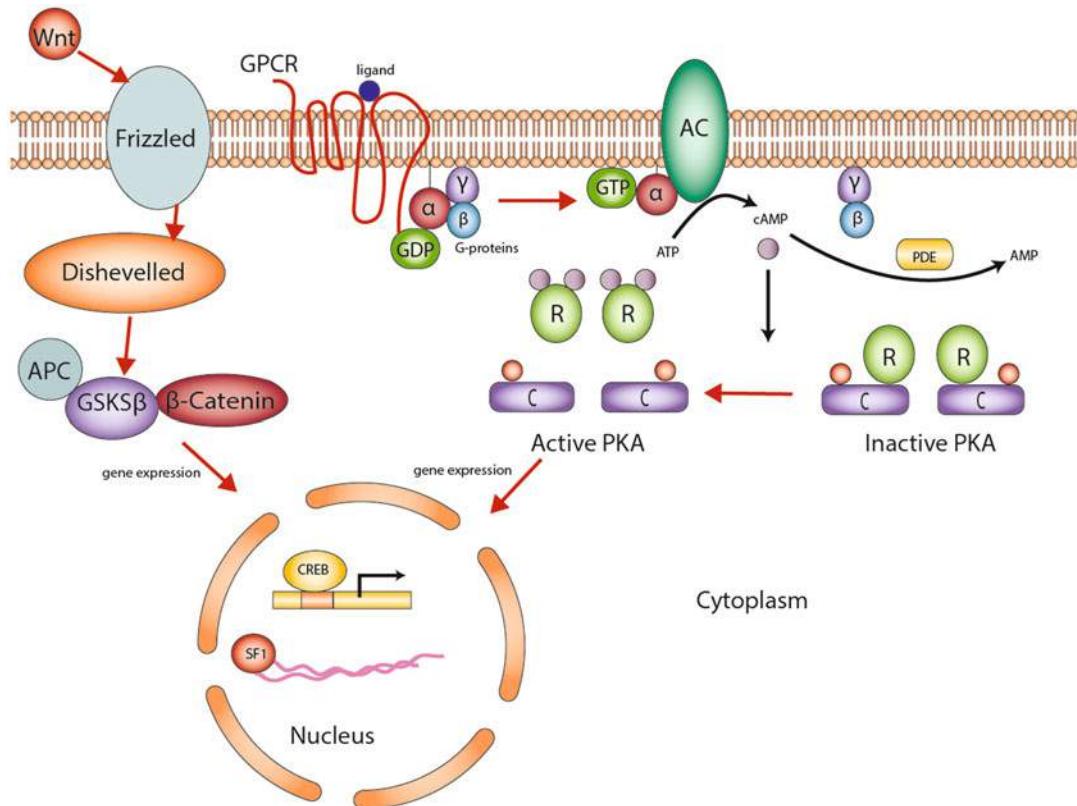


Fig. 3.1 The cAMP and *Wnt*-signaling pathways in benign adrenocortical tumors. ACTH activates MC2R, a seven-transmembrane receptor, which leads to activation of adenyl cyclase (AC) through $G\alpha$ subunit (encoded by *GNAS*). This step exchanges GDP for GTP, which in turn converts ATP into cAMP, activating protein kinase A (PKA). PKA is a holoenzyme that consists of a tetramer of two homo- or heterodimers regulatory subunits ($R1\alpha$, $R1\beta$, $R2\alpha$, and $R2\beta$) and catalytic subunits ($C\alpha$, $C\beta$, $C\gamma$, and PRKX); their dissociation in the presence of cAMP enables phosphorylation of PKA targets, including gene expression to mediate cell growth, differentiation, and hormone production (e.g., cortisol). *Wnt* ligand activates a series of Frizzled family receptors, which activates phosphoproteins that inhibit the phosphorylation of

β -catenin. The nuclear accumulation of β -catenin leads to the transcription of important genes, such as *WISP2*, *CTNNB1*, and *GSK3B*, as seen in PBMAH and PPNAD. Both signaling pathways share the downstream activation of certain oncogenic signals but differ substantially in their effects on others depending on the adrenocortical lesion. AC adenyl cyclase, C catalytic subunit of protein kinase A, cAMP cyclic AMP, CREB cyclic AMP response element binding protein, a transcription factor, GPCR G-protein-coupled receptor, $G\alpha$ stimulatory subunit α of the G-protein, *GSK3 β* glycogen synthase kinase 3 β , *PDE11A* phosphodiesterase 11A, PKA cAMP-dependent protein kinase, R regulatory subunit, SF1 steroidogenic factor 1, *WNT* wingless-type MMTV integration site family. Courtesy of Stratakis Laboratory, NICHD, NIH

Carney Complex (CNC)

Carney complex (CNC) is a hereditary multiple neoplasia syndrome with an autosomal dominant (AD) inheritance due to alterations in *PRKARIA* (17q22-24, CNC1 locus), an apparent tumor suppressor gene which encodes for the $R1\alpha$ subunit of PKA [14]. CNC is less commonly due to alterations of a yet-unidentified gene on chromosome 2p16 (CNC2) or *PRKACB* amplification. Over

one hundred germline inactivating mutations in $R1\alpha$ of PKA that are spread along the whole coding sequence, with the majority leading to a premature stop codon by nonsense or frame shift, have been described in approximately 80 % of CNC patients [15, 16]. The overall penetrance of CNC among *PRKARIA* mutations' carriers is above 95 % by the age of 50.

The clinical manifestations of CNC are broad. The main manifestation is CS from PPNAD

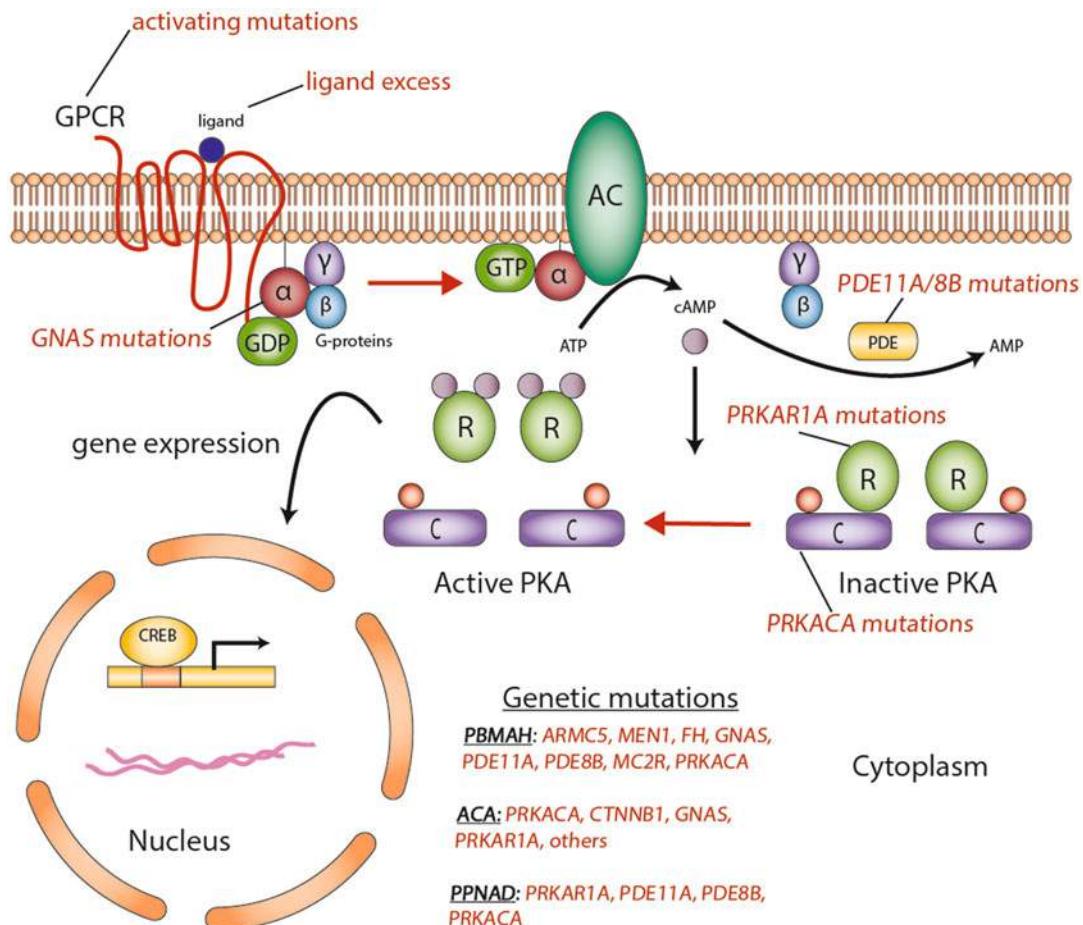


Fig. 3.2 Aberrations in the cAMP-dependent signaling pathways in benign adrenocortical tumors. The first alterations were reported in *GNAS* as seen in patients with MAS, followed by CNC through the inactivating mutations in *PRKAR1A* of PKA. This leads to constitutive activation of the pathway by increasing the availability of the PKA catalytic subunits. AC adenyl cyclase, C catalytic subunit of

protein kinase A, cAMP cyclic AMP, CNC Carny complex, CREB cyclic AMP response element binding protein, a transcription factor, GPCR G-protein-coupled receptor, *Gsa* stimulatory subunit α of the G-protein, MAS McCune–Albright syndrome, *PDE11A* phosphodiesterase 11A, PKA cAMP-dependent protein kinase, R regulatory subunit. Courtesy of Stratakis Laboratory, NICHD, NIH

in approximately 60 % of patients. Other tumors include cardiac myxomas, pigmented skin lesions (lentiginosis and blue nevi), somatotroph-pituitary adenomas, benign large cell calcifying Sertoli cell tumor (LCCSCT) of the testis, benign thyroid nodules, differentiated thyroid cancer, and melanocytic schwannomas. In-frame deletion of exon 3 and the c.708+1G>T mutation appears to confer a more severe CNC phenotype, while the splice variant c.709(-7-2)del6 and the initiation alternating substitution c.1A>G/p.M1Vp lead to an incomplete penetrance of CNC [17].

The hot spot c.491-492delTG mutation is most closely associated with lentigines, cardiac myxoma, and thyroid tumors as compared to all other *PRKAR1A* mutations. Usually, expressed RI α mutant protein present with more severe and aggressive CNC phenotype. Conversely, CNC2 occurs later in life with a lower frequency of myxomas, schwannomas, thyroid tumors, and LCCSCT. The clinical diagnosis of CNC is established if two or more of the aforementioned major manifestations exist. However, if a patient presents with a family history of CNC and one or

more of these manifestations, genetic testing will help establish the diagnosis.

Some patients may present with mild disease with i-PPNAD, with or without accompanied lentiginosis. This “subtype” of CNC is usually diagnosed before 8 years of age and may be due to pathogenic mutations in *PRKAR1A*, particularly c.709 (-7-2) del6 or c.1A>G/p.M1V, in approximately 50% of cases. Mutations in *PDE11A*, or *PDE8B*, have also been described and are detailed later. The diagnosis of i-PPNAD should be considered after a thorough exclusion of CNC, with close surveillance of the other possible manifestations of CNC as they may evolve with time.

Multiple Endocrine Neoplasia Type 1 (MEN-1)

Multiple Endocrine Neoplasia type 1 (MEN-1) is an AD syndrome due to a heterozygous inactivating germline mutation of the tumor suppressor gene *MEN1* (*menin*, 11q13) [18]. Mutations are found in approximately 90% of affected individuals with variable expression with age. Clinical features include a triad of primary hyperparathyroidism due to parathyroid hyperplasia (>95%), pituitary adenomas (45%), and neuroendocrine tumors (>30%). Other manifestations of MEN-1 include facial angiofibromas, collagenomas, carcinoid tumors, and meningiomas. Nonfunctional ACT are not uncommon in MEN-1 [19]. Gatta-Cherifi et al. reported adrenal enlargement in 20.4% (146/715) of patients with MEN-1, primarily due to macronodular ACT (10.1% of the cohort) [20]. Of the functional ACT, primary aldosteronism and adrenal CS predominate [20, 21].

Congenital Adrenal Hyperplasia (CAH)

Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive condition that leads to loss or decreased steroidogenesis due to various enzymatic deficiencies in the adrenal cortex. These

early defects affect the differentiation and zonation of the adrenal cortex. The most common defect in over 90% of cases is 21-hydroxylase deficiency (*CYP21A2*), a member of the cytochrome P450 family of enzymes [22]. Three types of 21-hydroxylase deficiency exist, and include the salt-wasting (most severe), simple virilizing and nonclassic types.

Patients with CAH are predisposed to ACT due to a compensatory increase in ACTH secretion from pituitary corticotrophs that promotes monoclonal proliferation of adrenal tissue. These lesions are often times benign and include adrenocortical adenomas, myelolipomas, and bilateral hyperplasia. One study found an increased prevalence of heterozygous *CYP21A2* germline mutations (large gene conversions for Q318X point mutations and intron 2 splice mutation) in patients with incidental ACT, when compared to the general population [23]. Another report showed a high incidence of nonfunctional ACT in approximately 82% in homozygous and 45% in heterozygous patients with CAH without a correlation between tumor size or serum 17-hydroxyprogesterone concentrations [24]. Moreover, *CYP21A2* mutation analysis appears to be the most reliable method for CAH diagnosis in the workup of incidental ACT [25]. Thus, CAH should be considered in the workup of ACT across all ages, as late-onset disease is not uncommon. A detailed classification of ACT in CAH is described in Chap. 11.

Familial Adenomatous Polyposis (FAP)

Familial Adenomatous Polyposis (FAP) is an AD disorder due to genetic defects in the tumor suppressor gene *APC* (5q22.2). The APC protein exerts numerous important activities in the cell including its proliferation, differentiation, and chromosome segregation. The presence of biallelic inactivation of *APC* (copresence of germline and somatic mutations) mediates the activation of the *Wnt/β-catenin* pathway, leading to tumorigenesis. The frequency of FAP is approximately 1 in 7500 in the general population.

FAP typically presents with large precancerous colorectal polyps in the second and third decade of life; extra-colonic manifestations include ACT, papillary thyroid carcinomas, lipomas, and pancreatic carcinomas. Various ACT and hyperplasia have been described in association with FAP, including nonfunctional ACA, ACC, and PBMAH, where germline and somatic mutations in *APC* were confirmed, albeit rare [26]. The risk of developing ACT in FAP is two to four times higher than the general population [27, 28].

Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC)

Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) is an AD disorder caused by inactivating mutations of the tumor suppressor gene fumarate hydratase (*FH*, 1q42.3-43). Biallelic inactivation of *FH* leads to increased tumorigenesis through the activation of the hypoxia-induced factor 1 (HIF1) pathway that results in alterations in glycolytic activity, neovascularization, and downregulation of apoptotic mechanisms in tumor tissue. These alterations predispose to hereditary leiomyomatosis, renal cancer, and ACT which are detected in approximately 8% of patients, such as PBMAH or isolated adrenal nodularity [29]. Loss of heterozygosity in the *FH* gene has been associated with PBMAH in patients with HLRCC only [29]. It seems plausible that *FH* may be a candidate gene for familial or sporadic PBMAH, which further expands the genetic spectrum of familial ACT.

Carney–Stratakis Syndrome (CSS)

Carney–Stratakis syndrome (CSS) is an AD disorder with incomplete penetrance that predisposes to the formation of gastrointestinal tumors (GIST), paragangliomas (PGL), and ACT [30]. The condition has been frequently referred to as the dyad of “paraganglioma and gastric stromal tumors.” Germline mutations in *SDHB* (1p36), *SDHC* (1q21), and *SDHD* (11q23) that were

known to be involved in inherited PGL and pheochromocytoma but were not previously involved in familial GIST or in ACTs have been linked to CSS. CSS may predispose to benign ACT, including PBMAH, albeit rarely.

Carney Triad (CT)

Carney triad (CT) is a sporadic condition with a female predominance that predisposes to hamartomatous lesions in various organs (pulmonary chondromas, pigmented, and other skin lesions), GIST, sarcomas, PGL, esophageal leiomyoma, and ACA. CT is the only known adrenal disease that has among its clinical manifestations adrenocortical and medullary involvement, such as coexisting PBMAH or ACA, and pheochromocytomas or PGL [31]. The genetics of CT has not been fully elucidated to date; however, it is known that CT is not due to *KIT*- or *PDGFRA*-activating mutation [32]. One study of 63 unrelated patients with CT found six patients (9.5%) with germline variants in the *SDHA*, *SDHB*, or *SDHC*, including loss of regions on the short arm (1p) and the long arm (1q) of chromosome 1 [33]. Unlike in CSS, patients with CT may harbor a recurrent aberrant dense DNA methylation at the gene locus of *SDHC* that leads to a reduced mRNA expression of SDHC and concurrent loss of the SDHC subunit on the protein level [34]. Since the vast majority of patients with CT have an unknown genetic defect, testing for *SDHA*, *SDHB*, or *SDHC* variations should be offered, as carriers may develop isolated adrenal disease and occasionally other tumors. Therefore, CT can be viewed as allelic to CSS in rare cases; however, in the majority of cases, epigenetic inactivation of the *SDHC* gene locus may be a plausible mechanism of tumorigenesis.

Familial Aldosteronism (FH)

Familial aldosteronism (FH) is a group of AD disorders that is estimated to affect approximately 2% of all patients with PA and is classified into three types. Type I (also known as

glucocorticoid-remediable aldosteronism; GRA) is an AD disorder characterized by a chimeric fusion of *CYP11B2* and *CYP11B1* (8q24.3), rendering the aldosterone synthase hybrid gene to be under the regulation of ACTH rather than the renin–angiotensin system [35]. This leads to increased production of aldosterone and hybrid steroids, such as 18-oxocortisol and 18-hydroxycortisol, which is suppressible by dexamethasone. However, significant phenotypic and biochemical heterogeneity exist, and some individuals may never develop hypertension. Benign ACT may be observed with GRA [36]. GRA should be considered in patients with early onset hypertension (<20 years) in the setting of a suppressed plasma renin activity, a family history of PA, or early cerebral hemorrhage (<40 years) from intracranial aneurysms or hemorrhagic strokes. Type II (7p22) typically affects adults and is characterized by hyperaldosteronism due to adrenocortical hyperplasia, an aldosterone-producing adenoma (APA), or both, that is not glucocorticoid remediable [37, 38]. Type III presents earlier, in childhood, with severe hypertension and metabolic derangements, and is due to a heterozygous mutation in *KCNJ5* (11q2) [39].

Genetic Aberrations in Adrenocortical Tumors

Adrenocortical Adenomas (ACA)

Adrenocortical adenomas (ACA) are the commonest form of solitary ACT and can be broadly classified as nonfunctioning or functioning. Approximately 10% of ACA are functioning and can be broadly divided into APA and CPA (detailed later). ACA are being identified with increasing frequency in approximately 5% of patients undergoing anatomic imaging for reasons other than adrenal pathology. Most ACA are small (<5 cm), well circumscribed, solid lesions that are histologically bright yellow due to their enriched cytoplasmic lipid. A subtype of ACT can be frequently seen with old age, hypertension, or diabetes mellitus and are referred to as nonneoplastic ACA and may present with domi-

nant, multifocal, or bilateral nodularity that are often times indistinguishable from other ACA. Thus, clinical, biochemical, and radiographic correlation are important in the subtyping of ACA.

The *Wnt/β-catenin* signaling pathway is activated in 25–50% of ACA, and in most cases due to mutations in *CTNNB1* (3p22.1). Moreover, loss-of-function mutations in *CTNNB1* have been identified in low frequency in females with APA, particularly in pregnancy (p.S33C, p.S45F, and p.G34C) at the target phosphorylation sites, with increased expression of aberrant receptors of luteinizing hormone, chorionic gonadotropin receptor (LH-CGR), and gonadotropin-releasing hormone receptor (GNRHR). APA tends to affect females before menopause and males over the age of 50. Males tend to harbor larger ACA, possibly related to the growth effects of sex steroid hormones on adrenal tissues from an unclear mechanism. Somatic allelic losses of *PRKAR1A*, leading to inactivation with resultant decreased PKA activity were reported in 23% of ACA [40]. Furthermore, aberrant GPCRs detected by in vivo stimulation tests appear to be common in these lesions [41].

Somatic mutations in the *KCNJ5* gene (11q24.3) have been implicated in the majority, 30–65%, of APA [42]. This gene encodes for the potassium ion channel Kir3.4 and localizes mainly to the zona glomerulosa (ZG) and to the outer part of zona fasciculata (ZF). Two hot spot somatic mutations on the highly conserved glycine–tyrosine–glycine (GYG) motif of the selective filter and the second transmembrane (TM) domain, p.G151R and p.L168R, are seen in >90% of tumors. Recently, germline mutations in *ARMC5* (16p11.2) have been implicated in a large number of PA. Zilbermint et al. identified germline mutations across the entire *ARMC5* gene in 39.3% of patients with APA. Interestingly, all mutant APAs affected patients of African Americans decent, which may explain their increased predisposition to PA. It appears that, in addition to the germline mutations, a second somatic variant is required in *ARMC5* to mediate tumorigenesis leading to polyclonal nodularity [43], as later explained in PBMAH. These

findings suggest that *ARMC5* plays an important role in the development of APA or other ACT, and may represent a new subtype of FH.

Other genes have been implicated in APA. The $\alpha 1$ subunit of Na⁺/K⁺ ATPase is encoded by *ATP1A1* (1p13.1), which is highly expressed in the adrenal cortex, and ZG. Two somatic substitutions (p.L104R, p.V332G) and one deletion (p.F100_L104) of *ATP1A1* were reported in about 8% of APA and are more common in males [44, 45]. The *ATP2B3* gene (Xq28) encodes for the calcium transporter ATPase 3 and is highly expressed in the adrenal cortex. In-frame deletions (between amino acid L424 and V429) have been implicated in about 1.5% of APAs, and particularly in females with a more severe phenotype [44, 45]. Additionally, several mutations in calcium channels have been implicated in APA, including *CACNA1D* (3p14.3), which encodes for the $\alpha 1$ subunit of L-type voltage calcium channel Cav1.3. Somatic (p.G403R and p.I770M) and de novo germline mutations (p.G403R and p.I770M) in *CACNA1D* are present in 3–11% of patients with APAs [46]. Unlike APA's due to *KCNJ5*, *CACNA1D* mutants contain a mix of ZG and ZF, and are more common in males. Germline mutations (p.M1549V) in another gene, *CACNA1H* (16p13.3), which encodes for the $\alpha 1$ subunit of T-type voltage calcium channel Cav3.2, were identified in early onset PA and may represent a new subtype of FH [47].

CPA is a benign subset of ACAs causing adrenal CS. Several genetic aberrations in the cAMP-dependent signaling pathway have been implicated in CPA, including somatic-activating mutations of *PRKACA* (c.617A>G/p.L206R) with an estimated incidence of approximately 42% (86 of 206 tumors studied to date) [48–50]. The sequence defects in *PRKACA* were present more significantly in younger patients with overt CS, suggesting a driver mutation role in tumorigenesis [51]. Somatic mutations in *GNAS* were identified in 5–17% of CPA [52]. The somatic allelic losses of *PRKARIA* were described in 23% of CPA; these tumors were smaller in size and had a paradoxical increase in urinary cortisol levels after dexamethasone suppression [40], due to increased glucocorticoid receptor expression

in ACT [53], as is often observed in patients with c-PPNAD. Defects in *Wnt* signaling have been reported in CPA, with *CTNNB1* (p.S45P, p.S45F) in approximately 23% of cases [54]. Other molecular events of adrenocortical tumorigenesis may be seen in CPA. One report described over-expression of *MC2R* in CPAs due to the human homolog of the Diminuto/Dwarf1 (hDiminuto) gene when compared to adjacent nontumorous adrenal tissue [55]. This gene is involved in steroid synthesis and cell elongation in plants, and further expands the genetic spectrum of CPA. However, there are still many unknown genetic defects that lead to CPA formation.

Genetic Aberrations in the cAMP-Dependent Signaling Pathway

Primary Bilateral Macronodular Adrenocortical Hyperplasia (PBMAH)

Primary Bilateral Macronodular Adrenocortical Hyperplasia (PBMAH) is a heterogeneous benign disorder characterized on the basis of hormone secretion, size, nodularity, and lipofuscin content, which determine pigmentation [56]. PBMAH is often associated with subclinical CS in adults over a number of years, accounting for less than 2% of all endogenous CS cases. Various biochemical patterns may be observed with PBMAH, including overt CS, cosecretion of aldosterone and cortisol (or its precursor steroids, and even estrogens), or aldosterone only [26, 57]. Cortisol secretion may be mediated by nonmutated aberrant receptors, such as gastrointestinal peptide (as seen with FDGS), vasopressin, serotonin, catecholamines, luteinizing hormone, or autocrine/paracrine ACTH, among other factors [58, 59]. These receptors are universally present in PBMAH, although their causative role in its development remains elusive [60]. Conversely, mutations of the *MC2R* gene have been reported in isolated cases of PBMAH [61]; MC2R and steroidogenic enzymes are also frequently reduced in PBMAH, which may explain in part the larger adrenal size as a compensatory mechanism.

Mutations in the tumor suppressor *ARMC5* gene were identified in over 50% of apparent

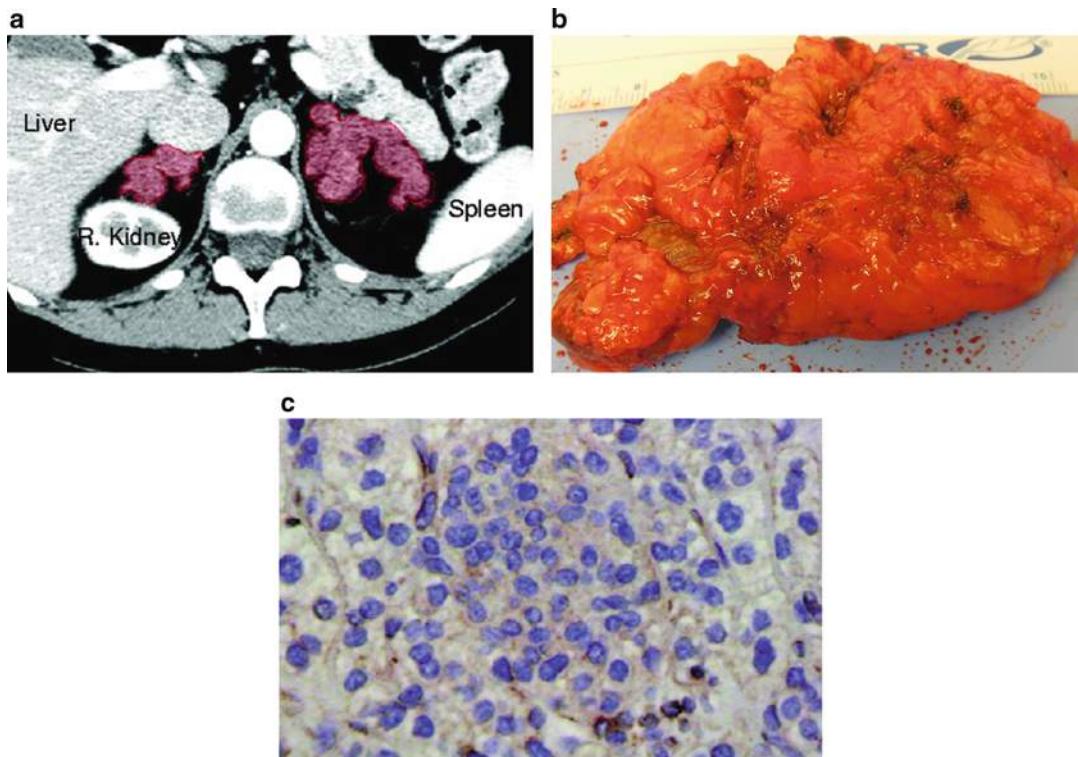


Fig. 3.3 Computed tomography (a), gross pathology (b), and ARMC5 immunohistochemical staining (c) of the left adrenal gland in a patient with primary bilateral macronodular adrenocortical hyperplasia (PBMAH). (From Correa et al. [43], with permission)

sporadic and familial PBMAH cases, where both alleles carried one germline and one somatic mutation each [62–64]. As in the case of APAs, a second somatic variant, either 16p loss of heterozygosity (LOH) or a second somatic mutation in the coding region of the gene, is required in addition to the germline mutation in *AMRC5* to mediate tumorigenesis leading to polyclonal nodularity [43]. The most frequent mutations at the germline or somatic level are frame shifts, stop codons, missenses, or deletions. Genetic variance of *ARMC5* is not uncommon; in one report, each of 16 ACT from PBMAH had a second new, “private,” and completely inactivating *ARMC5* defect, in addition to the germline mutation (Fig. 3.3) [43]. Patients with *ARMC5* mutations tend to have larger hyperplasia, with increased frequency of nodules, and severe hypercortisolism, likely due to the tumors loss of apoptotic mechanisms [65].

Other genetic aberrations in PBMAH have been described such as activating mutations at codon Arg (201) of *GNAS*, a substitution of histidine or serine, without features of MAS [26, 52], p.R867G *PDE11A* gene variant in one patient with familial PBMAH [26], and germline mutations in *FH*, *MEN1*, and *APC*, as previously detailed [26, 52]. In the majority of PBMAH cases, overexpression of the catalytic subunits of PKA, particularly in smaller nodules, without mutations in *PRKARIA*, suggests an important role for the kinases in tumorigenesis [66]. Conversely, germline duplications of *PRKACA* resulting in copy number gains have been reported in one family with PBMAH [48].

Larger ACTs in PBMAH accumulate an increased number of genomic/transcript abnormalities. Progression from smaller to larger lesions in PBMAH has been linked to chromosomes 20q13 and 14q23 [67]. However, the overexpression of

WISP2, *BCL2*, *E2F1*, *EGF*, *c-KIT*, *MYB*, *PRKACA*, and *CTNNB1* in PBMAH implicates various aberrant oncogenic pathways in nodular polyclonality and growth [67]. Furthermore, a recent report on PBMAH showed chromatin deregulation of *DOTIL* and *HDAC9*, which are genes known to be involved in regulating gene transcription and cell proliferation [49]. Collectively, the clinical spectrum and genetic aberrations in PBMAH are heterogeneous, and further studies are required to better elucidate the molecular mechanisms involved in its tumorigenesis.

Alterations of Phosphodiesterases (PDE)

Phosphodiesterases (PDE) are enzymes that function through the hydrolyzation of cAMP (PDEs isoform 4, 7, and 8) and cGMP (PDEs isoforms 5, 6, and 9) into their respective AMP and GMP [68, 69]. PDEs exist in over 100 isoforms and are derived from 21 genes separated into 11 *PDE* gene families [68, 69]. *PDE1*, *PDE2*, *PDE3*, *PDE10*, and *PDE11* possess dual specificity, acting on both cAMP and cGMP with varying affinities, depending on the isoform. The adrenal cortex expresses several isoforms of PDEs. *PDE2A* is the predominant adrenal isoform and has been implicated in the downregulation of aldosterone production in ZG cells, and the regulation of ACTH-induced increase in intracellular cAMP in ZF [70]. No studies to date have reported an association between alterations in *PDE2* and adrenocortical lesions, although *PDE2A* has been shown to be upregulated in *CTNNB1*-mutated ACT [71], as also observed in PPNAD due to somatic mutations in *CTNNB1* [72, 73].

More importantly, *PDE8B* and *PDE11A* have been implicated in ACT formation. *PDE8* comprises of two genes, *PDE8A* and *PDE8B*, encoding two highly specific enzymes responsible for the highest affinity to degrade cAMP [74]. Through negative modulation, these isomers play an important role in adrenal, ovarian, and testicular steroidogenesis [74, 75]. The first inactivating mutation in *PDE8B* (5q14.1) due to a novel missense mutation (c.914A>C, p.P305H) was reported in a 2-year-old girl with iMAD. Her

father carried the same genetic defect with subclinical disease [76]; the inheritance pattern was also seen in other alterations of PDEs [77, 78]. Several variations in *PDE8B* were found in samples including PBMAH, PPNAD, and functional/nonfunctional ACA [79]. The highly polymorphic *PDE11A* (2q31.2) encodes a dual-specificity PDE that degrades both cAMP and cGMP [74]. Horvath et al. reported three inactivating mutations in *PDE11A* in patients with PPNAD. In another study, the same group examined variations of *PDE* in ACT, hyperplasia, and the general population and found frequent missense mutations in *PDE11A* (p.R804H and p.R867G) among patients with ACT and the general population, with a lower frequency in adrenal hyperplasia (1.6%; $X^2=14.62$, $P<0.0001$) [78]. Variants of *PDE11A* were more frequent in patients with CNC due to *PRKARIA* mutations with a higher incidence of LCCSCT [80]. The mechanism by which partially inactivated *PDE* causes ACT is largely unknown. Collectively, genetic variations in *PDE8B* and *PDE11A* may be low-penetrance alleles (with a relative frequency in the general population), which may predispose for the development of ACT.

McCune–Albright Syndrome (MAS)

McCune–Albright Syndrome (MAS) is a heterogeneous disorder of mosaic populations of mutant and normal cells in affected organs that lead to polyostotic fibrous dysplasia, café-au-lait skin spots, precocious puberty, and overactive endocrinopathies. The first link of alterations in cAMP-dependent pathway in ACT and hyperplasia was reported in MAS. Affected individuals were found to have an increased predisposition of CS in the infantile period from nodular adrenal hyperplasia, mostly due to bimorphic pattern of diffuse and nodular hyperplasia (PBAD, a form of PBMAH) and a distinctive form of cortical atrophy with apparent ZG hyperplasia [81]. The genetic defect is due to a postzygotic gain-of-function point mutations in *GNAS* (also termed *gsp* mutations), within exon 8 of the *Gsα* subunit, which leads to constitutive activation of AC [82]. Clinical manifestations of MAS are highly variable and depend on the distribution of somatic

mosaic mutations in the various affected tissues. Patients with MAS may also present with a non-functional ACA at any age [83].

Isolated Micronodular Adrenocortical Disease (iMAD)

Isolated Micronodular Adrenocortical Disease (iMAD) represents a rare disorder of the adrenal glands of very early onset with multiple small yellow-to-dark brown nodules surrounded by a cortex with a uniform appearance. iMAD presents with moderate diffuse cortical hyperplasia with mostly nonpigmented nodules, attributed to mutations in *PRKARIA*, *PDE11A*, *PDE8B*, or germline duplications of *PRKACA*. The first inactivating mutation in *PDE8B* due to a novel missense mutation (c.914A>C, p.P305H) was reported in a 2-year-old girl with iMAD, where her father carried the same genetic defect with subclinical disease [76]. iMAD may be associated with a paradoxical rise of glucocorticoid excretion during the Liddle's test (1 mg overnight and low and high dose dexamethasone suppression tests) as also observed in patients with PPNAD [84].

Genetic Testing and Counseling of Patients with Benign Adrenocortical Tumors

Advances in the field of genetics are making screening and counseling of patients with ACT and hyperplasia an important clinical tool. Although there are no established genetic testing or counseling guidelines for affected individuals or carriers of mutations known to cause ACT, clinicians and genetic counselors should play a pivotal role in ensuring a patient's understanding of their condition. This is particularly important in today's medical practice, as the identification of genes responsible for the formation of ACT is being identified at increasing rates through genetic testing for reasons other than adrenal pathology. Many uncertainties about prognosis, morbidity, mortality, or the impact of a positive genetic screen on the individual or their families exist and remain an important area to explore in future studies.

Benign ACT is associated with increased morbidity and significant complications. Decreased lifespan and quality of life is primarily from increased cardiovascular risk. Early disease screening and intervention in affected or at-risk individuals may be associated with better outcomes. Genetic screening may begin as early as infancy in at-risk individuals, especially with conditions that can manifest with early mortality, such as CNS from cardiac myxomas or iMAD from MAS. Thus, a successful patient counseling model would incorporate patient's values and attitudes toward their disease, underscoring the risks and benefits of genetic screening and counseling, psychosocial interventions, and service delivery [85]. The most important component of the genetic counseling process in patients with adrenocortical disease is determination and communication of benign and malignant risk [85]. Other aspects of a successful counseling model include a thorough personal medical and family history from at least four generations (with a detailed family pedigree), education regarding the genetics of the condition, and discussions on prevention and screening options that should be carried out by an experienced genetic counselor.

Several genetic patterns of inheritance that lead to the formations of benign ACT exist (Tables 3.1 and 3.2). Mutations in the *SDH* sub-unit complex are inherited in an AD manner with age-dependent and incomplete penetrance. The *SDHD* gene shows a parent-of-origin effect, known as maternal imprinting. Germline mutations in *ARMC5* are inherited in an AD manner and should be considered in the majority of PBMAH with CS or PA, and particularly in the African American population. Family screening of *ARMC5*, as with the other genes in ACT and hyperplasia, will allow early detection of mutant carriers, and prospective follow up. Given the complexity of choosing between the multiple candidate genes with overlapping clinical phenotypes, testing genes either singly or in a panel, particularly in patients without known syndromic features, should be considered. Several sequencing technologies exists, such as next-generation sequencing or traditional Sanger sequencing, and analysis for larger structural defects such as deletions, translocations, or inversions, should be

Table 3.2 Familial syndromes associated with benign adrenocortical tumors

Familial syndromes	Gene (locus)	Mode of inheritance	Major features
Carney complex	<i>PRKAR1A</i> (17q22-24, CNC1 locus) <i>PRKACB</i> (2p16, CNC2 locus)	AD	<ul style="list-style-type: none"> • PPNAD • Cardiac myxomas • Pigmented skin lesions (lentigines and blue nevi) • Somatotroph-pituitary adenomas • LCCSCT • Benign thyroid nodules, differentiated thyroid cancer • Melanocytic schwannomas • ACT and rarely ACC
Multiple endocrine neoplasia type 1	<i>MEN1</i> (11q13)	AD	<ul style="list-style-type: none"> • Primary hyperparathyroidism • Pituitary adenomas • Neuroendocrine tumors
Congenital adrenal hyperplasia	<i>CYP21A2</i> (6p21.3) <i>CYP11B1</i> (8q24) <i>CYP17A1</i> (10q24.32)	AR	<ul style="list-style-type: none"> • Classic CAH: Salt-wasting (most severe), Simple virilizing • Nonclassic (late onset) • Others (refer to Chap. 11)
Familial adenomatous polyposis	<i>APC</i> (5q22.2)	AD	<ul style="list-style-type: none"> • Large precancerous colorectal polyps in the second and third decade of life • ACT, ACC • Papillary thyroid carcinomas • Lipomas • Pancreatic carcinomas
Hereditary leiomyomatosis and renal cell cancer	<i>FH</i> (1q42.3-43)	AD	<ul style="list-style-type: none"> • Hereditary leiomyomatosis • Renal cancer • ACT
Carney–Stratakis syndrome	<i>SDHB</i> (1p36) <i>SDHC</i> (1q21) <i>SDHD</i> (11q23)	AD	<ul style="list-style-type: none"> • GIST • Paragangliomas • ACT

(continued)

Table 3.2 (continued)

Familial syndromes	Gene (locus)	Mode of inheritance	Major features
Carney triad	Unknown genetic defect	Sporadic	<ul style="list-style-type: none"> Pulmonary chondromas Pigmented skin lesions GIST Sarcomas
	PGL		<ul style="list-style-type: none"> PGL Esophageal leiomyoma
	ACT		<ul style="list-style-type: none"> Type 1: severe early onset hypertension with significant phenotypic and biochemical heterogeneity exist, and some individuals may never develop hypertension. Should be considered in patients with early onset hypertension (<20 years) in the setting of a suppressed plasma renin activity, a family history of PA, or early cerebral hemorrhage (<40 years) from intracranial aneurysms or hemorrhagic strokes Type 2: affects adults and is characterized by hyperaldosteronism due to adrenocortical hyperplasia, an aldosterone-producing adenoma, or both, that is not glucocorticoid remediable Type 3: presents earlier, in childhood, with severe hypertension and metabolic derangements
Familial hyperaldosteronism	Type I (GRA): chimeric fusion of <i>CYP11B1</i> and <i>CYP11B1</i> (8q24.3) Type II: 7p22 Type III: heterozygous mutation in <i>KCNJ5</i> (11q2)	AD	<p>ACC adrenocortical carcinoma, ACT adrenocortical tumor, AD autosomal dominant, AR autosomal recessive, APC adenomatous polyposis coli gene, c-<i>PPNAD</i> CNC-associated PPNAD, CNC Carney complex, FAP familial adenomatous polyposis, FDGS food-dependent Cushing syndrome, GIST gastrointestinal stromal tumors, <i>GNAS</i> gene coding for the stimulatory subunit α of the G-protein (Gα), GPCR G-protein-coupled receptor, GRA glucocorticoid remediable aldosteronism, <i>HLRCS</i> hereditary leiomyomatosis and renal cancer syndrome, i-MAD isolated micronodular adrenocortical disease, i-<i>PPNAD</i> isolated PPNAD, LCCSCT benign large cell calcifying Sertoli cell tumor, <i>MAS</i> McCune-Albright syndrome, MEN1 multiple endocrine neoplasia type 1 gene, PBAD primary bimorphic adrenocortical disease, <i>PBMAD</i> primary bilateral macronodular adrenocortical hyperplasia, <i>PDE8B</i> phosphodiesterase 8B gene, <i>PDE1A</i> phosphodiesterase 11A gene, <i>PGL</i> paraganglioma, <i>PPNAD</i> primary pigmented micronodular adrenocortical disease, <i>PRKAR1A</i> protein kinase, cAMP-dependent, regulatory, type I, α gene</p>

considered, which may be missed with traditional testing. It should be noted that a significant number of patients with CNC might have *PRKARIA* haploinsufficiency due to genomic defects that are not detected by Sanger sequencing. In such circumstances, array-based studies are necessary for diagnostic confirmation of these defects [86]. Therefore, clinicians should consult a specialist in genetic testing before ordering such tests as a negative test does not exclude these conditions.

A number of factors are likely to affect the genotype–phenotype correlation of ACT. Such factors are not limited to developmental, hormonal, or gender-related differences. In the case of mutations in *PRKARIA* and *PDE11A*, allelic losses of the corresponding normal allele in adrenocortical tissues may also determine the phenotype [78]. Moreover, *PDE11A* is one of the genes associated with a low-penetrance predisposition to the development of ACT and is seen in high frequency in the general population [78]. Although the genotype–phenotype correlation is often times unpredictable, providing specific screening and counseling could decrease a patient’s anxiety toward this uncertainty, decrease genetic discrimination, and ensure appropriate disease surveillance.

Since hereditary tumors affect males and females of reproductive age, options of prenatal diagnosis with chorionic villus sampling or amniocentesis, pregnancy termination, or in vitro fertilization with preimplantation genetic diagnoses should be offered. One study found that knowledge of carrier status of *SDHx* mutations did not deter young couples/patients from having a desire to conceive in the future [85]. The complexity of these decisions, with their significant medical and psychosocial implications, is an important aspect of managing these patients and their families.

When dealing with a young patient with ACT, genetic counseling regardless of family history should be considered. Many of these conditions (e.g., germline mutations in *ARMC5* in PBMAH) have decreased penetrance and first-degree relatives that are carriers may not be affected. Therefore, all first-degree relatives with a known carrier state should be referred for genetic counseling. However, the clinician should bear in

mind that the occasional patient with sporadic ACT could also have a genetic mutation and have a low threshold for exploring genetic testing if the clinical phenotype warrants it. Thus, a physician should closely monitor any individual with a positive genetic test and no clear evidence of disease on physical examination or biochemical testing, as early detection of disease will lead to better outcomes. Further evaluation may be considered based on the type of mutation, tumor, stage, and/or patient’s clinical presentation.

Treatment of Benign ACT and Hyperplasia

Our knowledge on the diagnosis and management of ACT is evolving. Molecular defects in the cAMP and Wnt-signaling pathways that lead to the formation of ACT may be a potential for targeted therapy with pharmacologic inhibitors. However, preclinical and clinical studies are required to examine novel drugs targeting MC2R and PKA, for instance [87, 88]. Surgical resection of functioning ACT remains the standard of care.

Depending on the lesion in question, several surgical approaches may be considered. In PBMAH, subclinical hypercortisolism or modest cortisol secretion, defined as less than three times the upper limit of normal for the urinary free cortisol, predominates. In such circumstances, a laparoscopic unilateral adrenalectomy of the larger adrenal gland (or lesion) may be effective in inducing remission [89]. An experienced surgeon should perform the adrenalectomy as incomplete resection of PBMAH may occur owing to the large adrenal glands and multinodularity, which may extend below the mid pole of the kidneys. Postoperative adrenal insufficiency (AI) due to suppression of the contralateral adrenal gland from cortisol excess is expected in unilateral adrenalectomy; thus, a careful postoperative evaluation of AI and glucocorticoid replacement therapy in the perioperative period should be instituted (Table 3.3). In instances where PBMAH presents with PA only, preoperative evaluation for subclinical hypercortisolism should be considered as an undiagnosed subclinical CS may lead to AI in the perioperative period from contralateral adrenal

Table 3.3 Surgical approaches and perioperative glucocorticoid replacement therapy in patients with benign adrenocortical tumors

Adrenocortical lesion	Surgical approach	Perioperative glucocorticoid therapy
• CPA	• Unilateral adrenalectomy	<ul style="list-style-type: none"> • Can assume AI • 50 mg IV HC intraoperatively, then 25 mg IV every 8 h for 24 h • Decrease HC to 25 mg IV every 12 h on postoperative day 2 • If patient is doing well, tolerating oral intake, change to oral replacement dose of glucocorticoids: 10–12 mg/m² of body surface area
• APA with cosecretion of glucocorticoids and/or atrophy of contralateral adrenal gland on imaging	• BA	<ul style="list-style-type: none"> • As earlier • If unilateral adrenalectomy, perioperative glucocorticoids likely will not be necessary. Plan for ACTH stimulation test the following morning
• PPNAD	• BA	<ul style="list-style-type: none"> • If BA or presence of contralateral adrenal gland atrophy on imaging, assume AI and treat as detailed earlier. Of note, contralateral adrenal size does not correlate well with AI • If 18F-FDG PET/CT lateralizes to one adrenal gland, and/or a unilateral adrenalectomy will be performed, then assume that the other adrenal gland has been suppressed. Start perioperative glucocorticoids as earlier and plan for ACTH stimulation test the following morning^g • If 18F-FDG PET/CT lateralizes to both adrenal glands (or no significant signal in either gland), and BA is planned, start perioperative glucocorticoids as earlier
• PBMAH	• BA vs. unilateral adrenalectomy	<ul style="list-style-type: none"> • 18F-FDG PET/CT or AVS may help determine laterality of disease
• FDCS		
• iMAD		
• PBAD		
• Bilateral adrenal lesions		

AI adrenal insufficiency, *ACTH* adrenocorticotrophic hormone, *APA* aldosterone-producing adenoma, *AVS* adrenal vein sampling, *BA* bilateral adrenalectomy, *CBA* cortisol-producing adenoma, *FDCS* food-dependent Cushing syndrome, *HC* hydrocortisone, *i-MAD* isolated micronodular adrenocortical disease, *PPNAD* primary pigmented micronodular adrenocortical disease, *PBAD* primary bimorphic adrenocortical disease, *PBMAH* primary bilateral macronodular adrenocortical hyperplasia, *18F-FDG PET/CT* positron emission tomography with 2-deoxy-2-[fluorine-18]fluoro-d-glucose integrated with computed tomography

gland suppression, or adrenal crisis if left untreated. Therefore, it is imperative for patients with PBMAH, or other ACT, particularly if associated with clinical or subclinical CS, to undergo a thorough preoperative biochemical evaluation, preferably by an experienced endocrinologist, to help decrease morbidity and mortality associated with AI or adrenal crisis. Since laparoscopic adrenalectomy can be considered to confer a mild to moderate amount of “surgical stress,” and since typical individuals (not in AI) produce 25–75 mg cortisol in response, a safe glucocorticoid regimen during surgery is 50 mg of intravenous hydrocortisone, followed by 25 mg intravenous every 8 h for the first 24 h (see Table 3.3). Unlike PBMAH, bilateral adrenalectomy remains the standard of care for patients with iMAD or PPNAD. Radiography is unable to detect a larger adrenal gland in PPNAD with certainty, and lower postoperative remission rates of CS are seen with PPNAD if unilateral adrenalectomy is performed [90]. For the other lesions, including CPA and APA, unilateral adrenalectomy remains the standard of care, and perioperative glucocorticoid replacement therapy, particularly with CPA, or APA’s with concomitant cortisol cosecretion, should be initiated.

Several pitfalls exist in the evaluation of unilateral or bilateral adrenal lesions. It is important for the clinician to rule out concurrent autonomous cortisol secretion in the context of APA prior to adrenal venous sampling (AVS), for instance, to avoid misclassification of the culprit adrenal gland or lesion [91]. A functional mass in the context of bilateral adrenal lesions can be difficult to diagnose. One study examined the utility of positron emission tomography with 2-deoxy-2-[fluorine-18]fluoro-D-glucose integrated with computed tomography (18F-FDG PET/CT), a noninvasive diagnostic tool that provides tomographic images and can be used to obtain quantitative parameters concerning the metabolic activity of target tissues, in lateralizing a functioning mass in bilateral adrenal lesions. The authors reported a SUVmax cutoff of 5.33 with a 50.0% sensitivity and 81.8% specificity in localizing CPA [92]. Other studies have examined the use of AVS in lateralizing adrenocortical lesions, including the use of mass spectrometry (LC–MS/

MS) based steroid profiling in APAs or CPA [93]. Albeit preliminary, the use of 18F-FDG PET/CT or AVS in lateralizing adrenocortical lesions is promising.

Conclusions

The identification of several genetic alterations leading to the formation of benign ACT has paved our understanding of adrenocortical development and disease. Altered genes in the cAMP and *Wnt*-signaling pathways uncovered new molecular pathways, which have been implicated in the proliferation of adrenocortical cells, with potential therapeutic implications using novel therapies. However, large-scale clinical and molecular studies are underway, and needed, to further expand our understanding on the genotype–phenotype–biochemical correlations and genetic counseling of affected individuals and their families.

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Author and Contributors

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Conflict of Interest Statement

The authors declare that the research was conducted in absence of any potential conflict of interest.

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Guillaume Assié and Jérôme Bertherat

Adrenocortical cancer (ACC) is a rare tumor with an estimated incidence between 1 and 2 cases per million persons a year [1]. ACC is most often responsible for endocrine symptoms due to cortisol and androgen oversecretion, but it can also be diagnosed after investigations of symptoms related to tumor growth or after workup of an adrenal “incidentaloma.” The overall prognosis of ACC is poor, with a 5 years survival rate below 35 % in most series [2]. The idea that knowledge of the molecular genetics of this aggressive tumor could help to identify genetic predisposing factor, develop molecular tools for a better classification, and help to identify new therapeutic targets has prompted many progress in the field despite the rarity of this cancer.

Monoclonal tumors result from genetic alterations conferring a growth advantage on the cell initially affected. Initial studies by analysis of

the pattern of X-chromosome inactivation in heterozygous female tissue have shown that ACC are monoclonal, suggesting that an initial genetic or epigenetic alterations might occur in a single cell that acquire a selective advantage leading to tumor development [3, 4]. This initial observation was clearly a major stimulus to identify these molecular alterations. The genes involved in these molecular alterations could be classified as tumor suppressor genes on one hand, and oncogenes on the other hand. Molecular alterations would lead to inactivation of the tumor suppressor genes and activation of the oncogenes.

The genetics of ACC has been investigated initially by a candidate gene approach mostly based on the knowledge gained from the study of hereditary neoplasia syndromes in which adrenocortical tumors could be observed, like the Li–Fraumeni and Beckwith–Wiedemann syndromes but also multiple endocrine neoplasia type 1 or Familial Polyposis Coli. Subsequently, the study of the same genetic alterations in sporadic tumors led to the identification of various somatic alterations occurring during ACC development. More recently, the dramatic development of genomics tools has increased the rate of discovery of molecular alterations in ACC and by using this approach significant progress has been made this last decade. This review will summarize the finding on these three different aspects of the study of the molecular genetics of ACC.

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Hereditary Neoplasia Syndrome and Candidate Gene Approaches (Table 4.1)

Beckwith–Wiedemann Syndrome and IGF-II (Insulin-Like Growth Factor II) Gene Locus

The IGF-II gene located at 11p15 encodes an important fetal growth factor, is maternally imprinted, and is therefore expressed only from the paternal allele [5]. This 11p15 locus contains the IGF-II, H19, and the CDKNIC (p57kip2) genes. The H19 and p57kip2 genes are parentally imprinted and are therefore expressed from the maternal allele only. Genetic or epigenetic changes in the imprinted 11p15 region, resulting in increases in IGF-II expression, and mutations of the p57kip2 gene have been implicated in Beckwith–Wiedemann syndrome. This overgrowth disorder is characterized by macrosomia, macroglossia, organomegaly, and developmental abnormalities (in particular abdominal wall defects with exomphalos), embryonal tumors—such as Wilms' tumor—and ACC [6, 7], neuroblastoma, and hepatoblastoma.

IGF-II is involved in the development of the adrenal cortex [8–10]. Many studies have demonstrated that IGF-II is often strongly overexpressed in malignant adrenocortical tumors, with such overexpression observed in approximately 90 % of ACC [11, 12]. Transcriptome analysis of adrenocortical tumors has demonstrated that IGF-II is the gene most overexpressed in ACC by comparison with adrenocortical adenomas or normal adrenal glands [13–16]. The mechanisms underlying IGF-II overexpression are paternal isodisomy (loss of the maternal allele and duplication of the paternal allele) or, less frequently, loss of imprinting [17] (with maintenance of both parental alleles but a paternal-like IGF-II gene expression pattern) [12] (Fig. 4.1). Overexpressed IGF2 is thought to act in a paracrine manner through the insulin-like growth factor 1 receptor (IGF1R), sustaining tumor growth and cell proliferation.

The Li–Fraumeni Syndrome and TP53

The Li–Fraumeni syndrome (LFS) is an autosomal dominant disorder and confers susceptibility to breast carcinoma, soft tissue sarcoma, brain tumors, osteosarcoma, leukemia, and ACC [18]. The clinical criteria for LFS are usually a proband with bone or soft tissue sarcoma diagnosed before 45 years of age, a first-degree relative with cancer under 45 years of age and a first- or second-degree relative with any cancer before 45 years of age, or a sarcoma diagnosed at any age. The risk of cancer in LFS is estimated to be 50 % by 30 years of age and 90 % by 60 years of age. Germline mutations in *TP53* have been observed in 50–80 % of children with apparently sporadic ACC in North America and Europe [19, 20]. The incidence of pediatric ACC is about ten times higher in Southern Brazil than in the rest of the world, and a specific germline mutation has been identified in exon 10 of the *TP53* gene (R337H) in almost all cases [21, 22]. Molecular studies on this mutation have shown that the tissue-specific effects of this mutation may be pH dependent, due to the replacement of an arginine by a histidine in the tetramerization domain of TP53 [23]. The results of a systematic neonatal screening in the state of Paraná (southern Brazil) for the *TP53* R337R mutation have been reported on 171,649 newborns. This study is the first neonatal genomic DNA testing to select children for surveillance for a specific malignancy and suggest the benefit of this surveillance program to detect adrenocortical tumors before clinical signs develop [24]. In adults the frequency of *TP53* germline mutation is rather low, being reported between 3 and 6 % [25, 26]. However, this data suggests the discussion of genetic testing and screening for *TP53* mutation in young adults may be justified.

In sporadic ACC in adults, by a candidate gene approach somatic mutations of *TP53* have been reported in 25 % of cases, mostly within exons 5 and 8 [27–29]. Loss of heterozygosity (LOH) at the *TP53* locus (17p13) has been consistently demonstrated in ACC but is much less

Table 4.1 Genes associated with multiple neoplasia genetic syndromes and adrenocortical tumors

Genes, chromosomal location and type of alteration TP53 (17p13)	Associated genetic disease and MIM reference number Li–Fraumeni syndrome	Tumors and nontumoral manifestations associated with germline defect Soft-tissue sarcoma, breast cancers, brain tumors, leukemia, ACC (3 %)	Somatic genetic defect observed in apparently sporadic adrenocortical tumors TP53 somatic mutations in sporadic ACC (30 %) 17p13 LOH in sporadic ACC (>80 %)
Menin (11q13)	Multiple endocrine neoplasia type 1	Parathyroid, pituitary, pancreas tumors, adrenal cortex (25–40 %), among which are ACA, hyperplasia, and rare ACC (<1 %)	Very rare somatic menin gene mutations in sporadic adrenocortical tumors Frequent 11q13 LOH in ACC (90 %)
11p15 (IGF2) locus alterations p57kip2 (CDKN1C) (genetic defect) KCNQ10T (epigenetic defect) H19 (epigenetic defect)	Beckwith–Wiedemann syndrome	Omphalocele, macroglossia, macrosomia, hemihypertrophy, Wilms tumor, ACC (3 %)	ACC: 11p15 LOH (>80 %) ACC: IGF2 overexpression (>80 %)
APC (5q12–22)	Familial adenomatous polyposis coli	Multiple adenomatous polyps and cancer of the colon and rectum. Possible extracolonic manifestations include perianalpillary cancer, thyroid tumors, hepatoblastoma, rare cases of ACC, ACA, multiples or bilateral ACA Congenital hypertrophy of the retinal pigment epithelium also occurs	Transcriptome analysis shows Wnt-signaling activation in AIMAH, PPNAD, and ACC β-catenin somatic mutations in ACA and ACC (30 %)

ACA adrenocortical adenoma, ACC adrenocortical cancer, AIMAH adrenocorticotrophic-hormone-independent macronodular adrenal hyperplasia, APC adenomatous polyposis coli, IGF2 insulin-like growth factor 2, LOH loss of heterozygosity
This table describes genetic diseases associated with ACC and the other associated tumors and nontumor manifestations. The first three columns show the genetics of various familial syndromes. In the third column, manifestations are listed in order of prevalence, with adrenal disorders highlighted in bold. The last column describes alterations of the same genes or loci observed in apparently sporadic adrenocortical tumors

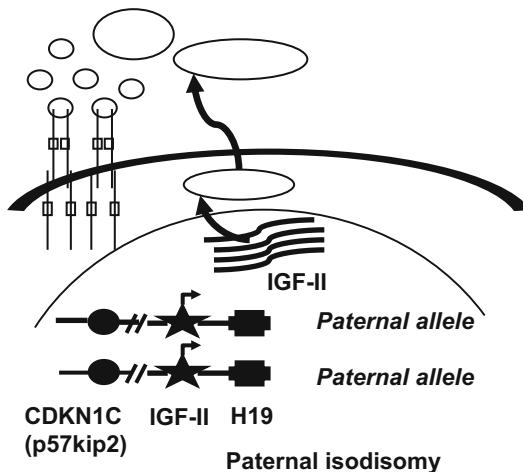


Fig. 4.1 11p15 and IGF2 overexpression in adrenocortical cancer

frequent in adrenocortical adenomas [10]. In an early attempt to develop diagnostic molecular marker, 17p13 LOH was demonstrated to be an independent variable predictive of recurrence after complete surgical removal of localized ACC [10].

Lynch Syndrome and DNA Mismatch-Repair Genes

The Lynch syndrome (LS) is an inherited disorder that increases the risk of many types of cancer, particularly colorectal cancer. LS is caused by germline mutations in DNA mismatch-repair (MMR) genes MLH1 (mutL homolog 1), MSH2 (mutS homolog 2), MSH6 (mutS homolog 6), and PMS2 (postmeiotic segregation increased 2). LS patients also have an increased risk of cancers of the endometrium, ovary, thyroid, lung, small intestine, liver, central nervous system, skin, and adrenal cortex. Karamurzin et al. first reported four cases of ACC in patients with Lynch syndrome [30]. Recently, Raymond et al. reported three ACC patients with a family history suggestive of LS [31]. Mutations in the MMR gene were

found in the three families. Patients with ACC and a personal or family history of LS tumors could be also considered for genetic testing and screening for ACC.

Multiple Endocrine Neoplasia Type 1 and Menin

The tumor suppressor gene *MEN1* is located at the 11q13 locus. A heterozygous inactivating germline mutation of *MEN1* is found in about 90% of families affected by multiple endocrine neoplasia type 1 (MEN 1). The principal clinical endocrine tumors observed are parathyroid (95%), endocrine pancreas (45%), and pituitary (45%) tumors [32]. Adrenocortical tumors and/or hyperplasia are observed in 25-40% of MEN 1 patients [33]. In most cases, they are nonfunctional adrenocortical adenomas that can be managed conservatively with radiological/hormonal follow-up. ACC has rarely been reported in MEN 1 patients; however, a recent study reported ACC in 8 out of 715 patients. Mutation in *MEN1* by candidate gene approach and specific sanger sequencing was found to be rare in adrenocortical tumors [34, 35]. By contrast, LOH at the *MEN1* locus (11q13) were identified in more than 90% of informative ACC in three different series whereas it has been reported in less than 20% of adrenocortical adenomas [34-36]. LOH in ACC involves almost the entire 11q domain.

β-Catenin and the Wnt-β-Catenin Signaling Pathway

The Wnt signaling pathway plays a major developmental role in various tissues, including the adrenal cortex. It is normally activated mainly during embryonic development and β -catenin is a key component of this pathway. It has a structural role in cell-cell adhesion and is a transcription cofactor with T cell factor/lymphoid enhancer

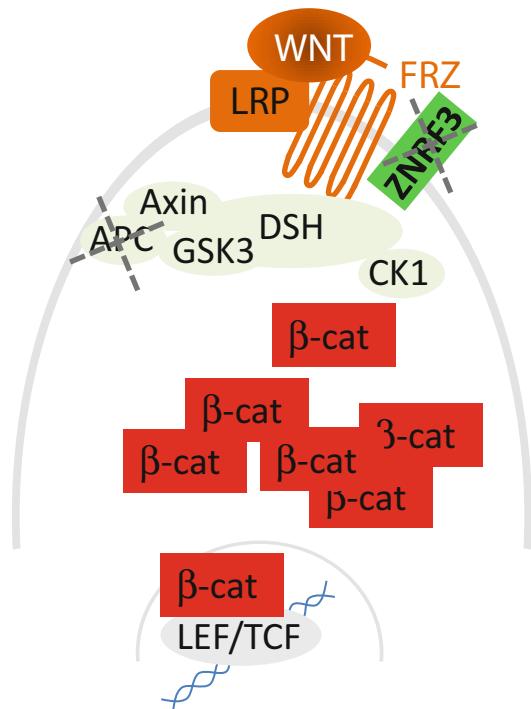


Fig. 4.2 Activation of the Wnt/β-catenin signaling pathway in adrenocortical cancer

factor (TCF/LEF) mediating transcriptional activation of target genes of the Wnt signaling pathway [37] (Fig. 4.2).

From Familial Adenomatous Polyposis Coli (FAP) to *CTNNB1* (Coding for β-Catenin) Mutations in Adrenal Tumors

Genetic alterations of the Wnt signaling pathway were initially reported in familial adenomatous polyposis coli (FAP), and later on in a series of other malignancies [38]. Individual cases of patients with adrenocortical tumors in the context of familial adenomatous polyposis coli were also reported [39]. Furthermore, familial adenomatous polyposis coli patients with germline mutations of the APC (Adenomatous Polyposis Coli) gene that lead to an activation of the Wnt signaling pathway may develop adrenocortical tumors [40].

These evidences led Tissier et al. to study specifically β-catenin in ACC [41]. They showed

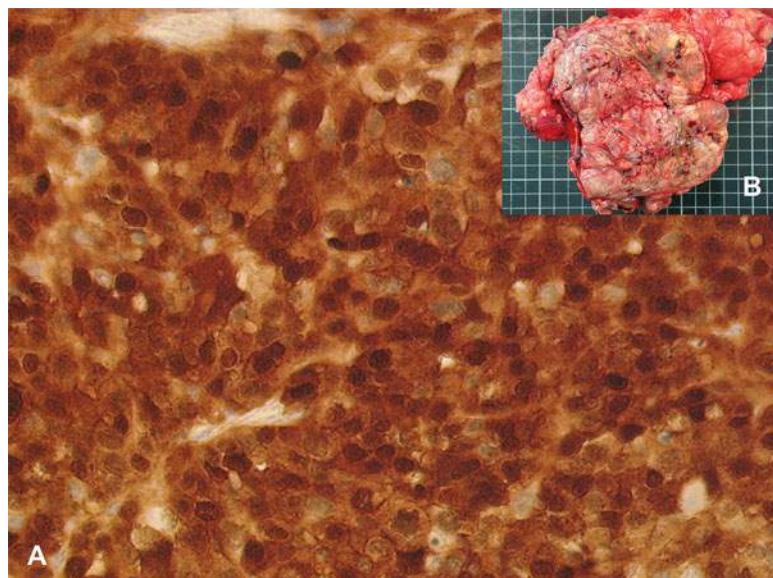
an abnormal β-catenin localization and genetic alterations in a series of 13 ACC. Immunohistochemistry revealed an abnormal cytoplasmic and/or nuclear accumulation of β-catenin in 11 of 13 ACC samples (Fig. 4.3). Activating somatic mutations of the β-catenin gene *CTNNB1* was found in 4 of these 13 tumors, and they were associated with abnormal β-catenin accumulation [41]. This prevalence of around one quarter of sporadic ACC was later confirmed by genomic studies (see next section). Of note, these mutations were also found in around one quarter of adrenocortical adenomas and in other benign adrenal tumors [41–44]. All these mutations in exon 3 of the β-catenin gene affected specific serine and threonine residues, and amino acids adjacent to them, which are essential for the targeted degradation of the β-catenin protein [38].

Role of β-Catenin Activation in Adrenocortical Tumors Development

The H295R cell line is the most commonly used cell model of human ACC. Interestingly, the H295R cell line presents an activating β-catenin mutation. In these cells the Wnt/β-catenin pathway is activated, as ascertained by constitutive activation of T-cell factor-dependent transcription [41]. Doghman et al. showed that Wnt/β-catenin inhibitor, PKF115-584, inhibited proliferation of the H295R human ACC cell line [45]. By flow cytometric analysis, they showed that PKF115-584 precludes H295R cells from entering cell division and increases apoptosis.

The finding of *CTNNB1* mutations in ACC and in benign adrenal tumors raised the question of a potential multistep tumor progression from benign to malignant adrenocortical tumors. Moreover, some rare adrenocortical tumors in which a malignant and a benign zone are associated within the same adrenal gland are consistent with this hypothesis [46, 47]. However, the high frequency of adrenocortical adenoma, which are usually incidentally discovered, contrasts with the rarity of ACC, suggesting that this multistep progression from benign to malignant tumors, if true, seems to be very rare.

Fig. 4.3 β -catenin immunohistochemistry of an adrenocortical cancer. *CTNNB1* activating mutation induces diffuse cytoplasmic staining and nuclear accumulation of β -catenin (a). Macroscopic aspect of the tumor (b)



Alternatives to *CTNNB1* Mutation for Wnt Activation in ACC

Activation of the Wnt/ β -catenin pathway in ACC can currently be explained in about a quarter of the tumors by somatic *CTNNB1* mutations, coding for β -catenin. However, immunohistochemical and gene profiling studies suggest a more prevalent activation of this pathway. Among candidate genes in this pathway, *APC* mutations have been reported in ACC, but seem rare [48, 49]. More recently, with single nucleotide polymorphism (SNP) array and exome sequencing, *ZNRF3* mutations and homozygous deletions were identified as one of the most frequent somatic events in ACC. *ZNRF3* has been reported to be a negative regulator of the Wnt/ β -catenin pathway. It has therefore been suggested that *ZNRF3* is a new tumor suppressor gene and an alternative to *CTNNB1* mutations for activating this pathway (see next section).

Genomics of ACC

Since 2004, more than 40 original studies focused on some aspect of ACC genomics, including transcriptome, miRNome, methylome, chromosome alterations, and exome sequencing. More recently, two consortia reported an integrated

overview of ACC, performing all these omics in single sets of ACC, one released by the European Network for the Study of Adrenal Tumors (ENSAT) consortium and the French Ligue Contre Le Cancer (Tumor Identity Card program) [50], and the other by The Cancer Genome Atlas (TCGA) ACC consortium [51].

Transcriptome

Almost 15 original studies [13, 15, 16, 50–61] have focused on the transcriptome of ACC (Table 4.2). The first transcriptome study comparing ACC and adrenocortical adenoma (ACA) was published in 2003 [13]. All these studies are based on DNA microarray, except the latest, recently released by TCGA, which is based on RNA sequencing [51]. A majority of these studies are in good agreement regarding tumor classification and underlying transcriptome signatures (Fig. 4.4).

The Malignancy Signature

The most striking feature of adrenocortical tumors transcriptome is the ability to discriminate benign from malignant tumors. Indeed, this discrimination is possible using transcriptome-based classification tools, including principal

Table 4.2 Transcriptome analyses of adrenocortical cancers

Study	Main conclusions of the authors	Transcriptome platform	Main analytical methods	ACC (N)	Other sample types (N)
Giordano et al. [13]	91 Genes show >3-fold expression difference in ACC vs. normal tissue and ACA.	Affymetrix HG_U95Av2 ~10,000 genes	Unsupervised classification	11	ACA (4), MH(1), NA (3)
de Fraipont et al. [15]	Up in ACC: IGF2, SPP, STK15, TOP2A, Ki-67 2 clusters of genes—the IGF2 cluster (8 genes) and the steroidogenesis cluster (14 genes)—predict malignancy. A set of 14 genes (including PTGB2, GZMA, and ATF1) predicts metastatic recurrence.	Custom array with 230 cDNA clones	Group comparison (malignant vs. benign)	24	ACA (33)
Velázquez-Fernandez et al. [52]	Up in ACC: IGF2, TGF β 2, FGFR1, FGFR4, MST1R, TGFBR1, KCNQ1OT1, GAPD Down in ACC: StAR, CYP11A, HSD3B1, CYP11B1, CYP21A2, CYP17	Ultra GAPS slides (Corning, Corning, NY) with a QArray (Genetix, New Milton, UK) ~10,000 genes	Supervised classification (hierarchical clustering on a set of selected genes)	7	ACA (13)
Slater et al. [53]	42 genes show >4-fold expression difference in ACC vs. normal tissue, and 21 genes in ACC vs. ACA	IMT H. sapiens cDNA 11.5k Chip	Unsupervised classification	10	ACA (10), NA (10)
Lombardi et al. [54]	Up in ACC: IGF2 4 genes show a >1.5-fold expression difference in ACC vs. ACA Up in ACC: HSP-60, CCND1, TOP1	Down in ACC: CGB, EGR-1 Atlas Technologies cDNA expression arrays 82 genes	Group comparison	7	ACA (7)

(continued)

Table 4.2 (continued)

Study	Main conclusions of the authors	Transcriptome platform	Main analytical methods	ACC (N)	Other sample types (N)
Fernandez-Ranvier et al. [55]	25 genes located on chromosome 1 1q13 are downregulated in ACC vs. ACA	Affymetrix U133 plus 2.0 Analysis restricted to the 314 genes located in 11q13	Group comparison	11	ACA (43)
Fernandez-Ranvier et al. [56]	37 Genes show a >8-fold expression difference in ACC vs. ACA. Among these, the 5 genes below show the highest ability to discriminate ACC vs. ACA:	Affymetrix U133 plus 2.0-47,000 transcripts, ~20,000 genes	Group comparison (malignant vs. benign)	5	ACA (74)
Tömböl et al. [57]	Up in ACC: IL13RA2, CCNB2 Down in ACC: HTR2B, RARRES2, SLC16A9 Up in ACC: TOP2A, IGF-2, CCNB2, Down in ACC: CDKN1C CDC2, CDC25C	44K Whole human genome microarrays (Agilent Tech. Inc) ~44,000 transcripts	Group comparison (malignant vs. benign)	7	ACA (19), NA (10)
Soon et al. [58]	177 genes show expression difference in ACC vs. ACA	Affymetrix U133 plus 2.0-47,000 transcripts, ~20,000 genes	Unsupervised AND supervised classifications	12	ACA (16), NA (6)
Giordano et al. [59]	Up in ACC: IGF2, MAD2L1, and CCNB1 Down in ACC: ABILIM1, NAV3, SEPT4, RPRM 1890 genes show a 1.5-fold-expression difference in ACC vs. ACA. Clustering analysis reveals 2 subtypes of ACC, associated with different outcome and different mitotic count ACC subgroup 1: high mitotic grade ACC subgroup 2: low mitotic grade	Affymetrix U133 plus 2.0-47,000 transcripts, ~20,000 genes	Group comparison (malignant vs. benign) Unsupervised classification Survival analysis	33 33 (10)	ACA (22), NA (10)

de Reyniès et al. [16]	Clustering analysis discriminates ACC from ACA, and reveals 2 subtypes of ACC, C1A, and C1B, associated with different outcome. Molecular predictors of survival are proposed Disease-free predictor: based on RT-qPCR expression of DLG7 and PINK1	Affymetrix U133 plus 2.0-47,000 transcripts, ~20,000 genes	Unsupervised classification (hierarchical clustering) Survival analysis	35	ACA(57)
Laurell et al. [60]	Clustering analysis discriminates ACC from ACA, and reveals 2 subtypes of ACC associated with different outcome Up in ACC: IGF2, FGFR1 and FGFR4, USP4, UBE2C, UFD1L	RT-qPCR expression of BUB1B and PINK1 Ultra GAPS slides (Corning, Lowell) ~30,000 transcripts ~19,000 genes	Unsupervised classification (hierarchical clustering) Survival analysis	11	ACA (17), NA (4)
Assié et al. [50] [16]	ACC are classified in C1A and C1B, according to de Reyniès 2009 ACC adrenocortical adenoma, ACC adrenocortical cancer	Affymetrix Human Gene 2.0 ST arrays	Unsupervised classification 44 (13 new; 31 common with de Reyniès [16])	44 (13 new; 31 common with de Reyniès [16])	0
Gara et al. [61]	Clustering analyses clearly discriminate ACC from ACA and normal adrenals. ACC signature is mostly related to genes downregulated	Affymetrix U133 plus 2.0-47,000 transcripts, ~20,000 genes	Unsupervised classification	20	75
Zheng et al. [51]	ACC are classified in 4 groups, depending on the “steroid phenotype” signature (high or low) and the proliferation signature (high or low) ACA adrenocortical adenoma, ACC adrenocortical cancer	Illumina RNA sequencing	Unsupervised classification	78	0

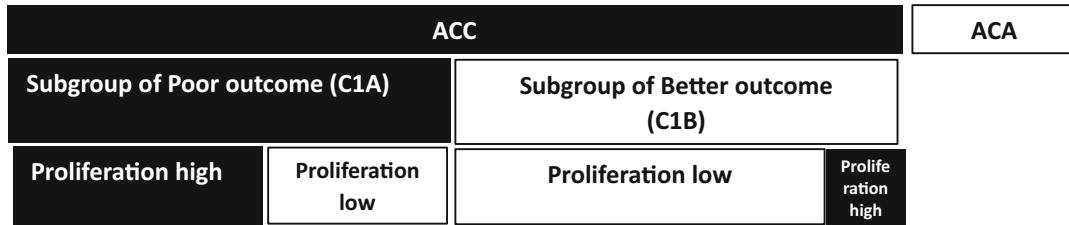


Fig. 4.4 General classification of adrenocortical cancer, based on unsupervised transcriptome classification

component analysis, or hierarchical clustering, potentially with consensus clustering strategies [62]. To feed these classifiers, some authors focused on selected genes, with a priori knowledge of the differential expression between benign and malignant tumors. However, other studies considering all genes with no a prior selection also showed discrimination between benign and malignant tumors. It even appears that the malignant/benign signature is the strongest transcriptome signature. Two meta-analyses have also confirmed this consistent feature [63, 64].

Several thousands of genes are differentially expressed between ACC and ACA. These genes can be called a malignancy signature. This signature corresponds to two distinct categories of genes: a general proliferation signature, that is common to all cancer types, and a signature specific to adrenocortical tumors.

The general proliferation signature is mainly composed of cell cycle regulators and cell cycle effectors [65]. Among others, the proliferation signature includes cyclin-related genes, with an apparent enrichment of genes involved in G1/S transition. Other proliferation genes are related to chromosome remodeling, other aspects of mitosis, including DNA replication, chromosome segregation, and apoptosis-related genes.

The adrenal-specific malignancy signature includes overexpression of specific growth factor pathways, among which is the insulin-like growth factor II (IGF2) pathway. Several transcriptome analyses confirmed IGF2 overexpression in a majority of ACCs and also demonstrated that among growth factors, IGF2 is the most overexpressed gene (see Table 4.2). Other members of the IGF2 pathway are expressed in ACC, including

IGF1 receptor –which mediates the growth effects of IGF2 [66] and IGF2-binding proteins. It appears that IGF-I receptor, which mediates the trophic effects of IGF2, is expressed at the same level in benign and malignant tumors. Several studies demonstrated in vitro the importance of IGF2 pathway for adrenal proliferation [67, 68]. Several other growth factors or growth factors receptors are overexpressed in ACC, including for instance FGFR1 or FGFR4 (see Table 4.2), but their functional relevance is not clearly established.

Two Major Molecular Subtypes of ACC with Different Outcomes

Using unsupervised classification strategies, several studies identified two different groups of ACCs, related to different transcriptome profiles [16, 50, 51, 59]. Most remarkably, these two groups are distinct in terms of outcome, one group being of poor outcome, the other one reported of better outcome. In one series [16], 5-year survival was 91 % in the good prognosis group and 20 % in the bad prognosis group (log-rank *p*-value <0.05). In terms of pathology, the Weiss malignancy score is higher in the poor prognosis group, owing mainly to a higher proliferation rate. Giordano et al. linked the transcriptome groups to proliferation, by the ACC grade (high grade versus low grade) [59]. However, pathology does not perfectly reflect transcriptome. Indeed some tumors with low Weiss score and low proliferation index are observed in the poor prognosis group, and in contrast high Weiss score and high proliferation can occasionally be observed in the better outcome group.

Tumors from the poor prognosis group also tend to be more often associated with metastases. However, survival prediction based on transcriptome remained significant after stratifying on tumor extension [16].

A few thousands of genes discriminate these two groups. The poor prognostic group is associated with a nonspecific proliferation signature, with enrichment in mitotic cell cycle related genes, including for instance CDK6, cell division cycle 2G1 to S and G2 to M and cyclin B2 [16, 59]. The most recent transcriptome study identified more specifically a significant enrichment in genes related to steroidogenesis in the poor prognosis group, whereas the better outcome group was enriched in genes related to inflammation [51].

Further Dividing ACC into Subgroups

The most recent transcriptome study was performed using RNA sequencing on 78 ACC [51]. The authors proposed to further classify ACC in subgroups, depending on the intensity of the proliferation signature. This now divides the poor prognostic group into one of high proliferation, and the other of lower proliferation signature. Of note in the group of better outcome, the authors identified a high proliferation signature in 2 ACCs, corresponding to unusual forms of undifferentiated ACC (sarcomatoid ACC) [51]. This finding recapitulates previous works showing that overlap between poor and better outcome transcriptome subgroups did not overlap completely with proliferation index.

miRnome

MicroRNAs (miRNAs) are small double-RNA (20 nt) strands, regulating gene expression at posttranscriptional level. MiRNAs targets specific mRNA molecules by sequence complementarity [69]. Targeted mRNAs are reduced, thus inducing a lower gene expression. There are nearly 2000 miRNAs. Some of these miRNAs can be good biomarkers for cancer diagnosis and prognosis [70]. Nine main studies have focused on the miRNA expression profile in ACC [50, 51,

57, 61, 71–75] (Table 4.3), using different techniques: cDNA microarray, quantitative PCR, or miRNA sequencing.

MiRNA Malignancy Signature

As for mRNA, miRNA studies demonstrate that unsupervised classification based on miRNA expression profile discriminate ACC from ACA (see Table 4.3). This classification is sustained by miRNAs differentially expressed between ACA and ACC. Among those miRNAs, a few are robust, that is, reported in a majority of studies. These include overexpression of miR-483-5 (see Table 4.3). Interestingly, this miRNA is expressed from the IGF2 locus, one of the genes with the strongest expression in ACC. Its expression correlates positively with the expression of IGF2. Among consensus miRNA with low expression, miR-195 and miR-335 are often reported among other miRNAs (see Table 4.3).

Subgroups of ACC

Three studies identified miRNA signatures, discriminating ACC with poor or better outcome [50, 74, 75]. However, no single miRNA signature has emerged so far. Some sets of miRNAs, known as clusters of miRNAs, are coded from loci close to each other, and expressed globally together. Using miRNA sequencing, the ENSAT study reported a cluster of miRNA called DLK1-MEG3, on chromosome 14q32.2, specifically downregulated in a subgroup of ACC with good outcome [50]. This cluster of miRNAs is submitted to parental imprinting, with specific expression from the maternal allele. In this series, loss of expression was related to LOH, with loss of the mother allele. This series reported another cluster of miRNAs, the miR-506-514 cluster, on chromosome Xq27.3, with low expression in ACC of poor outcome compared to ACC of better outcome [50]. However, these results were not confirmed in the recent TCGA paper. This discrepancy may be either related to statistical issues—sample sizes not large enough, or to technical issues as the miRNAs were not prepared in the same way. Further studies will be needed to clarify these points.

Table 4.3 miRNome analyses of adrenocortical cancers

Study	Main conclusions of the authors	Genomic technique	Main analytical methods	ACC (N)	Other sample types (N)
Soon et al. [71]	The miRNome discriminates ACC from ACA. 23 miRNA show expression difference in ACC vs. ACA. ACC with low miR-195 and high miR-483-5p have a worse survival	miRCURY LNA array v10.0 (Exiqon)	Unsupervised classification (hierarchical clustering)	22	ACA (27), NA (6)
Tömböl et al. [57]	Up in ACC: miR-483-5p Down in ACC: miR-335 and miR-195 6 miRNA show expression difference in ACC vs. ACA and normal adrenal. ACC can be discriminated from ACA with miR-511 and miR-503 expression measurement	~850 miRs	Group comparison		
Patterson et al. [72]	Up in ACC: miR-184 and miR-503 The miRNome discriminates ACC from ACA. 23 miRNA show expression difference in ACC vs. ACA. The miR-483 locus is within the IGF-2 locus. Their expressions are correlated	Down in ACC: miR-511 and miR-214	Supervised classification (based on the genes differentially expressed)	4	ACA (8), NA (4)
Schmitz et al. [73]	Up in ACC: miR-483-5p The miRNome discriminates ACC from ACA. 248 miRNA show expression difference in ACC vs. ACA. Low expression of miR-675 and miR-335 is associated with malignancy	~400 miRs	Group comparison		
Özata et al. [74]	Down in ACC: miR-100, miR-125b, and miR-195 The miRNome discriminates ACC from ACA. Seventy-two miRNA showed expression difference in ACC vs. ACA. High expression of miR-503, miR-1202, miR-1275 is associated with a poor prognosis	~2000 mRNA	Unsupervised classification (hierarchical clustering)	10	ACA (26), NA (21)
	Up in ACC: miR-483-3p, miR-483-5p, miR-210, miR-21 Down in ACC: miR-195, miR-497, miR-1974	~700 miRNA	Supervised classification (hierarchical clustering on a set of selected genes)	4	ACA (9), NA (4)
			Group comparison	22	ACA (26)
			Survival analysis		

Chabre et al. [75]	12 miRNAs show expression difference in ACC vs. ACA. 29 miRNA show expression difference between aggressive ACC (metastatic or recurring within 3 years) and nonaggressive ACC (nonrecurring after 3 years). Combinations of these miRNA discriminate ACC from ACA and aggressive ACC from nonaggressive ACC. Among these miRNA, some can be assayed in the serum. These circulating miRNA have a diagnostic and prognostic value Up in ACC: miR-483-5p Up in aggressive ACC; miR-139-5p, miR-376a	miRExplore™ Microarrays (Millenyl Biotec, Bergisch Gladbach, Germany)	Group comparison	12	ACA (6)
Assié et al. [50]	3 groups of ACC were identified, based on the expression profile of 2 clusters of miRNA: Cluster miRNA-506-514 cluster: 11 miRNA on chromosome Xq27.3, overexpressed in C1B ACC vs. C1A (cf de Reyniès JCO 2009)	Cluster DLK1-MEG3 cluster: 38 miRNA on chromosome 14q32.2, underexpressed in a subset of C1B ACC	miRNA sequencing (Illumina)	45	0
Zheng et al. [51]	6 Groups of ACC identified, not related to outcome ACA adrenocortical adenoma, ACC adrenocortical cancer	miRNA sequencing (Illumina)	Unsupervised classification	79	0

Beyond miRNAs: A Complex Biology

MiRNAs main function is to downregulate targeted genes [69]. Predicting these targets is not easy, because the targets are not only defined by sequence complementarity. Especially it has been demonstrated that targets depend on the cell type and on cell differentiation. Thus, target confirmation requires *in vitro* validation—in cell culture—for each miRNA. Therefore, there is no exhaustive evaluation of genes positively or negatively regulated by miRNAs in ACC to date.

Another fascinating biological aspect of miRNAs is the distant action of some miRNAs, secreted by some cells, and acting in distant cells through paracrine effect. Circulating miRNAs have been identified in ACC [75, 76]. Their potential biological action remains to be determined.

Exome Sequencing

Advances in next-generation sequencing (NGS) allow sequencing of the entire genome. It is now possible to identify the mutations occurring in tumors by comparing the tumor sequence to leukocyte or normal tissue sequence from the same patient.

A Limited Set of ACC Driver Genes

Sequencing the whole exome of ACC has been reported in three main publications [50, 51, 77]. In these works, ACC exome was compared to germline (leukocyte or nontumor adjacent tissue). Almost 20 genes have been identified as recurrently mutated in ACC (Table 4.4). The first report in 2014 focused on 45 ACC, by the ENSAT consortium [50]. Exome sequencing was combined to SNP arrays, another genotyping technique convenient for detecting homozygous deletions and high-level amplifications. The cohort was expanded to 122 ACC, including 77 additional ACC tested by targeted NGS and SNP array. The targeted NGS was designed to include all genes seen at least 3 of the 45 tumors. One of the most commonly altered genes was *ZNRF3*, an E3-ubiquitine ligase, never identified in cancer before this study. Juhlin et al. reported in 2015

another analysis of 41 ACC exome, using a similar strategy [77] (Fig. 4.5). In 2016, the TCGA consortium reported a series of 90 ACC analyzed by exome sequencing, RNA sequencing, and also by SNP array [51]. Mutated genes included the majority of genes in previous publications, but included also new genes, adding to the list of recurrently mutated genes. Two other studies used NGS to characterize the mutations in ACC, analyzing the genetic variation of a limited number of cancer-related genes, and restricting the analysis to the tumor DNA [78, 79].

The most striking feature is that a limited number of less than 20 genes are recurrently altered in ACC (see Table 4.4). Based on their recurrence, these genes are considered as drivers of ACC tumorigenesis. These driver genes are mutated in ~50% of ACCs. Of note, the other half of ACCs do not show any alteration in these genes. Mutations do occur in this latter group of ACC, but mutations are not recurrent (“private” mutations}. Among driver genes identified by ACC exome sequencing, *ZNRF3* is one of the most commonly altered genes, found in more than 20% of cases. *ZNRF3* has been reported to be a negative regulator of the Wnt/β-catenin signaling pathway [80]. In ACC, *ZNRF3* is inactivated, either by mutation, but mainly by homozygous deletions. This suggests that *ZNRF3* inactivation is a way to induce Wnt/β-catenin signaling in ACC. This pathway is also activated directly by *CTNNB1* activating mutations—encoding the β-catenin-, found in ~15% of ACC. Interestingly, *ZNRF3* and *CTNNB1* mutations are mutually exclusive in ACC (see Fig. 4.5). Other recurrently mutated genes are related to cell cycle regulation. These genes include *TP53* mutations in ~15% of ACC, tumor suppressors *CDKN2A* and *RBL*, ribosomal protein *RPL22*, and the oncogenes *MDM2* and *CDK4*. Alterations of these genes are commonly found in cancer, and globally dysregulate the cell cycle, one of the key elements of cancer proliferation [50, 51].

Chromosome maintenance is also crucial for cancer cells. Indeed, *TERT* and *TERF2* amplifications are recurrent in ACC. A few *TERT* promoter mutations have also been identified in ACC [51, 81]. Recurrent mutations are also

Table 4.4 Somatic mutations identified by exome sequencing of adrenocortical cancers [50, 51, 77]

Gene	Pathway	Function	Type of alteration	Frequency (%)
ZNRF3	Wnt/β-catenin	E3 ubiquitin ligase, negatively modulates the Wnt/β-catenin pathway by promoting LRP5/Frizzled receptor turnover. Loss of expression induces an activation of the Wnt/β-catenin pathway	Inactivating mutations and homozygous deletions	~20
CTNNB1		Encodes β-catenin, key regulator of the Wnt/β-catenin pathway. Activation induce abnormal activation of this pathway	Activating mutations	~15
APC		Important negative regulator of Wnt/β-catenin pathway. Inactivation induces abnormal activation of this pathway	Inactivating mutations	<1
KREMEN1		Membrane receptor. When activated, negatively regulates Wnt/β-catenin	Homozygous deletions	~1
TP53	Cell cycle, TP53/Rb	Encodes p53, key positive regulator of apoptosis, cell-cycle arrest, DNA repair	Inactivating mutations	~20
CDKN2A		Tumor suppressor genes encoding two proteins, acting through the activation of p53 and pRB	Inactivating mutations and homozygous deletions	~10
CDK4		Oncogene, inhibiting pRB (encoded by RB1) by phosphorylation	Activated by high-level amplifications	~5
MDM2		E3 ubiquitin ligase, negatively regulates p53 protein by leading it to its proteasomal degradation	High-level amplification	~1
RB1		Encodes pRB, negative regulator of cell cycle	Inactivating mutations and homozygous deletions	~7
CCNE1		Oncogene, regulating G1/S transition	Activated by high-level amplification	~3
RPL22		Encodes 60S ribosomal protein L22. Related to MDM2-mediated p53 ubiquitination and degradation	Inactivating mutations	~3

(continued)

Table 4.4 (continued)

Gene	Pathway	Function	Type of alteration	Frequency (%)
TERT	Chromatin remodeling/ telomere maintenance	Reverse transcriptase of telomerase complex. Need for maintaining the chromosome length in cancer cells	Activated by high-level amplification	~10
TERF2		Component of the telomerase complex	Activated by high-level amplification	~5
MEN1		Regulates transcription by coordinating the chromatin remodeling	Inactivating mutations	~7
DAXX		Implicated in chromatin remodeling, in alternative lengthening of telomere and in apoptosis	Inactivating mutations	~2
ATRX		Implicated in chromatin remodeling, and in alternative lengthening of telomere	Inactivating mutations	~1
MLL4		Histone modifying enzyme	Inactivating mutation	~3
PRKAR1A		Negative regulatory subunit of protein kinase A. Inactivation induces PKA activation through de-repression	Inactivating mutations	~5
MED12		Component of the transcription initiation complex	Inactivating mutation	~3
NF1		Negative regulator of RAS signaling pathway	Inactivating mutations and homozygous deletions	~5

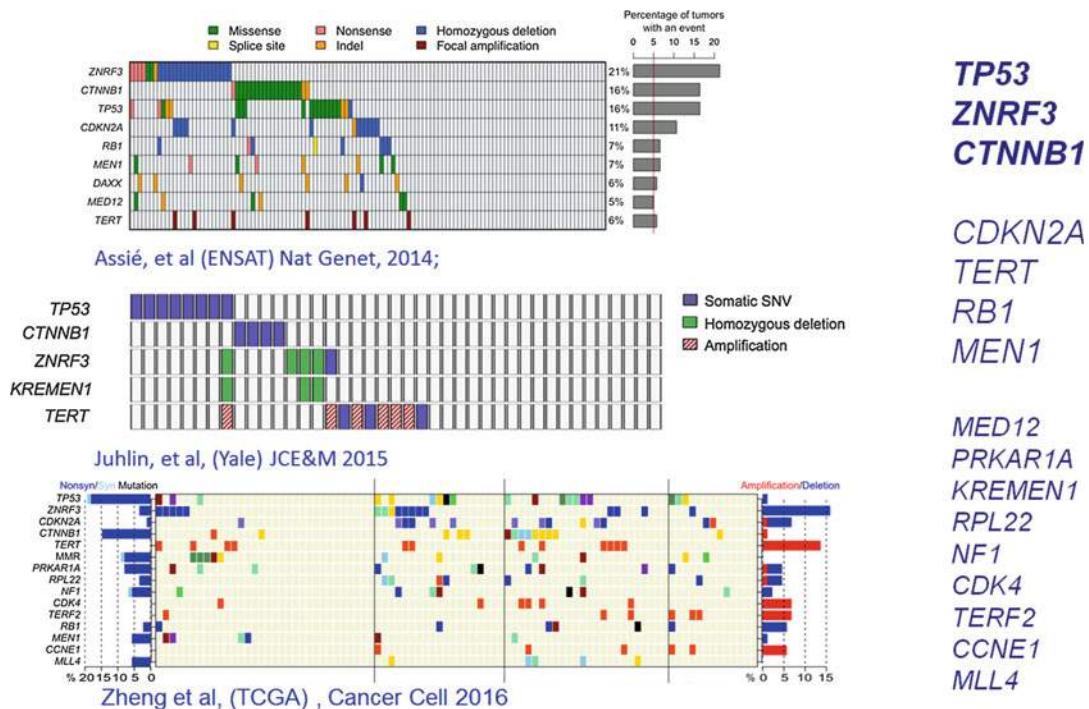


Fig. 4.5 Recurrently mutated genes in adrenocortical cancer identified by exome sequencing

found in genes related to chromatin remodeling, with mutations of genes already identified in neuroendocrine tumors (*MEN1*, *DAXX*, *ATRX*), but also histone modifiers. In addition, telomere maintenance is crucial in cancer cells, and amplification of *TERT* or *TERF2*—one component of the telomerase complex—is present in ACC. Of note, in ACC with *DAXX* or *ATRX* mutations, alternative lengthening of telomeres—corresponding mechanisms of telomere maintenance independent from *TERT*—can be observed [50, 51].

Recurrent mutations in *PRKAR1A* have also been reported in ACC [51]. *PRKAR1A* encodes for the regulatory subunit of PKA, a negative regulator of the cAMP/PKA pathway. cAMP/PKA pathway activation is central in benign adrenal tumors secreting cortisol. Germline *PRKAR1A* mutations predispose to Primary Pigmented Nodular Adrenal Dysplasia (PPNAD), responsible for Cushing syndrome [82]. Therefore, these cases of ACC with *PRKAR1A* mutations raise the question of the potential role of

cAMP/PKA pathway in ACC, and subsequently of the potential of benign tumors transforming into ACC. However, *PRKAR1A* mutations are reported as late somatic events in at least half of tumors [51].

Finally other many genes are altered, with no recurrence (“private” mutations). The global importance of these genes in ACC pathophysiology remains to be clarified.

Beyond the Genes: General Features of Mutagenesis in ACC

The median somatic mutation rate was reported between 0.6 and 0.9/Mb, comparable to pancreatic adenocarcinoma, but twice as much as thyroid cancer [50, 51, 77]. This mutation rate is variable and globally correlates with prognosis (the higher the mutation rate, the worse the prognosis). Of note, a few instances of hypermutated tumors (>10 mutations/Mb) have been identified among ACCs. Hypermutation was not associated with prognosis [50, 51].

The majority of mutations in ACC are C>T transitions, as is found in most solid tumors [50, 51]. Considering the nucleotide context around mutations, mutational signatures can be identified [83]. The most common signature, Signature 1 (a C>T substitution in the CG context), evokes an age- and DNA-mismatch repair-deficiency signatures. This signature is shared with a majority of gastrointestinal cancers. Another signature, Signature 4 (a C>A substitution in the CG context), evokes a smoking signature, as is shared with adenocarcinomas and squamous cell lung cancer. Another signature, Signature 2 (a more complex combination of substitutions), is also common in ACC. No clear cause is related to this mutation [51].

The Drivers of Pediatric ACC

One publication by Pinto et al. reported in 2015 the genomic alterations in a cohort of 37 pediatric ACC [84]. Three driver genes could be identified. The most commonly mutated gene is *TP53*, found in 28/37 patients (76 %), including 25 with germline mutations (12/25 presented the Brazilian hotspot R332H) and 3 somatic mutations.

ATRX, a gene implied in chromatin remodeling, was the second most mutated gene, found in 32 % of ACC samples. *ATRX* mutations were associated with alternative telomere lengthening. Three patients with ACC had somatic *CTNNB1* mutations. Combining *TP53* and *ATRX* mutational status resulted in a 3 group classification—both mutated, *TP53* only mutated, and no mutation—with distinct outcome—worse, variable, and better prognosis, respectively.

Chromosome Alterations

Chromosome alterations include copy number changes, LOH, chromosome structure alterations, and alterations of ploidy. Copy number changes in ACC have been studied by comparative genomic hybridization (CGH) [85–89] (Table 4.5) and then by comparative CGH arrays [63, 90, 91]. More recently, ACC chromosomal alterations were analyzed by SNP arrays, focusing on LOH and ploidy beyond copy number [50, 51].

Chromosomal Alterations Are Numerous in ACC

In comparison to ACA, ACC are characterized by an accumulation of numerous chromosomal alterations. This was first reported by studies based on conventional CGH [85–89]. These studies have identified numerous chromosomal alterations, including a majority of gain—mainly in chromosomes 5, 7, 12, and 17, and some chromosomal losses—mainly chromosome 1p, 2q, and 3. However, the resolution of these studies is low, and the results are sometimes inconsistent (e.g., loss of 8q in 3 studies but not in others). In CGH array profiling studies, more consistent profiles were observed [63, 90, 91] (see Table 4.5). Specifically, recurrent gains in chromosome 5, 7, 12, 19, and losses of chromosome 22 were reported. This profile differs significantly from that in ACA, the latter showing much fewer chromosomal gains or losses (see Table 4.5).

Subgroups of ACC

Two studies specifically focused on chromosomal alterations using SNP arrays [50, 51] (see Table 4.5). Subsets of ACC with specific alteration profiles could be identified. A first original profile was the finding of extended LOH, affecting more than half of the chromosomes, with a set of chromosomes almost always affected—including chromosomes 1, 2, 6, 11, 13, 14, 15, 17, 18, and 22 (Fig. 4.6) [50, 51]. This group of ACC was named “Chromosomal” ACC in the TCGA study [51]. This LOH was related to chromosome losses in half of ACCs harboring this profile. By losing a large part of their genome, these tumors are not diploid anymore; they do not have 2 pairs of chromosomes, but are, rather, hypodiploid. However, another half of ACCs with this extended LOH do not have chromosome loss. Actually these latter tumors seem to correspond to a later stage of the latter: some hypodiploid ACC replicate their genome, thus becoming diploid but with keeping this extended LOH. A few of these ACCs even replicate further their genome, becoming polyploid, but still with these large ranges of LOH. Of interest, genome replication in “chromosomal” ACC is associated with poorer outcome, compared to “chromosomal” ACC with no replication [51].

Table 4.5 Chromosome alterations identified by comparative genomic hybridization (CGH) and SNP arrays

Study	Main conclusions of the authors	Genomic technique	Main analytical methods	ACC (N)	Other sample types (N)
Zhao et al. [85]	Putative role of SAS/CDK4 and MDM2 coamplification (12q) in the progression of adrenocortical tumors	CGH array (Amplionco™ I Microarray)	Mapping of alterations restricted to 58 regions of the genome (descriptive)	5	ACA (4)
	Gains in ACC: 5q, 12q, 20q	174 P1, PAC, or BAC probes 58 regions targeted			
Stephan et al. [90]	Several recurrent gains and losses are identified in ACC (see later). Several isolated CGH probes scattered among the genome are associated with survival. The combination of these probes identifies 3 groups of ACC with different survival	Agilent 44K Human Genome CGH array	Mapping of alterations (descriptive)	25	0
	Gains in ACC: 5, 7, 12, 16q, 20 Losses in ACC: 1, 3p, 10q, 11, 14q, 15q, 17, 22q		Survival analysis		
Szabó et al. [63]	Several recurrent gains are identified in ACC (see below)	Agilent 44K Human Genome CGH array	Mapping of alterations (descriptive)	4	0
	Gains in ACC: 5, 7, 12, 19q	CGH array (IntegraChip, IntegraGen)	Mapping of alterations (descriptive)	52	ACA (86)
Barreau et al. [91]	A larger proportion of the genome is altered in ACC vs. ACA. A qPCR-based diagnostic tool is proposed. The chromosome alterations also contain a prognostic information	~4,400 BAC probes	Survival analysis		
	Gains in ACC: 5, 7, 12, 16, 19, Losses: 13, 22 and 20				
Assié et al. [50]	Two different types of ACC can be identified: some ACC with extended LOH, and other with numerous subchromosomal alterations. Extended LOH is related to hypodiploidy, with potentially subsequent replication of the remaining genome	SNP array (Illumina)	Mapping of alterations (descriptive)	120	0
de Martino et al. [78]	Several recurrent gains are identified in ACC, in agreement with Stephan et al [90] and Barreau et al. [91]	CGH Array 180k (Agilent)	Mapping of alterations (descriptive)	28	0
Zheng et al. [51]	Three different types of ACC can be identified: “chromosomal” ACC with extended LOH, “noisy” ACC with numerous subchromosomal alterations, and “quiet” ACC with reduced number of alterations. “Noisy” ACC are associated with a worse outcome	SNP array (Affymetrix SNP6)	Mapping of alterations (descriptive) survival analysis	89	0

ACA adrenocortical adenoma, ACC adrenocortical cancer, *LOH* loss of heterozygosity

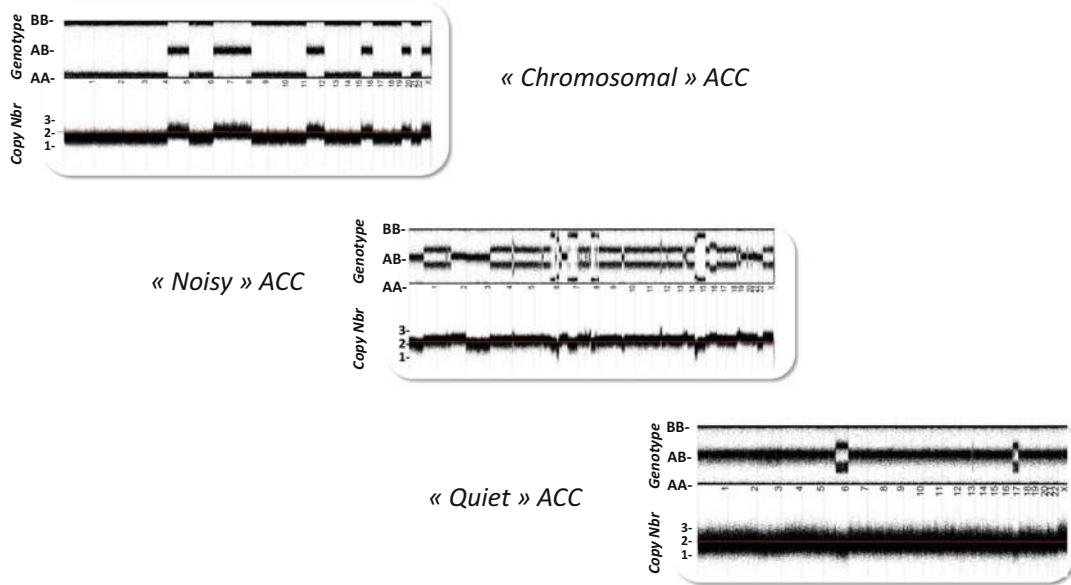


Fig. 4.6 Single nucleotide polymorphism (SNP) array profiling shows three different adrenocortical cancer (ACC) profiles: “chromosomal,” ACC with extended loss of heterozygosity, “noisy,” ACC with many subchromo-

somal alterations, and “quiet,” ACC with a reduced number of chromosome alterations. AA, AB, and BB: SNP genotypes (AB are heterozygous, AA and BB are homozygous); Copy Nbr: DNA copy number

Another group of ACC do not harbor these extended ranges of LOH, but instead harbor numerous chromosomal alterations [50, 51] and were thereafter named “noisy” ACC (see Fig. 4.6). Interestingly, noisy ACC are associated with a worse prognosis, compared to “chromosomal” ACC. Finally, it was recently reported that a few ACC do not harbor many chromosome alterations, thus named “quiet” ACCs [51] (see Fig. 4.6).

Pathophysiological Consequences of Chromosome Alterations

DNA copy-number alterations may impact gene expression level. Especially, high-level amplification is associated with oncogene activation. Such high-level copy number gains are rare in ACC, affecting mainly chromosome 12 (including the gene *CDK4*) and chromosome 5 [50, 51, 78, 91]. Common focal chromosome gain is observed at the *TERT* locus, coding for Telomerase. These recurrent gains may contribute to maintain *TERT* expression, and thus to maintain chromosome length. Another gene

related to telomerase, called *TERF*, is also often gained in ACC [50, 51]. Pathophysiological consequences of specific patterns of chromosome alterations—e.g., “chromosomal,” “noisy,” and “quiet” profiles—remain to be determined.

DNA Methylation

Epigenetic refers to heritable changes in gene expression that occur independently of changes in primary DNA sequence. Among those, DNA methylation seems to have a critical role in the transcriptional regulation and may lead to various diseases including cancer. DNA methylation of cytosines by DNA methyltransferase enzymes occurs mainly in CpG dinucleotides. Large clusters of CpG dinucleotides, called CpG islands, are enriched mostly in the promoter regions. Two types of changes in DNA methylation patterns can be observed in cancer: first, a global hypomethylation inducing genomic instability, loss of parental imprinting, and reactivation of transposable elements and alternatively, and second,

hypermethylation of CpG islands located in the promoter regions of tumor suppressor genes [92].

DNA methylation can be studied globally using DNA microarrays, targeting cytosines potentially methylated. The general principle is to treat DNA with bisulfite, before DNA array genotyping. By doing so, methylated cytosines remain cytosines, whereas unmethylated cytosines become uracile. Thus, genotyping either a cytosine or an uracile will indicate cytosine methylation. Five recent studies have focused on genome-wide DNA methylation profiling in ACC (Table 4.6) [50, 51, 93–95].

Global DNA Methylation in ACC

Rechache et al. studied 450,000 DNA regions, with a majority in intergenic regions. They could show that ACC were globally hypomethylated compared to ACA [95]. In addition, using unsupervised classification, they could show that methylome is able to discriminate ACC from ACA, and metastases from primary tumors.

CpG Island Methylator Phenotype (CIMP) in ACC

Focusing levels in CpG islands from gene promoter regions, Barreau et al. showed that methylation actually vary among ACC, some showing methylation levels comparable to ACA, while others were hypermethylated [50, 93]. The hypermethylation profile of some cancers has been reported in other malignancies, and referred to as CIMP for “CpG island methylator phenotype.” This was confirmed in an independent cohort [51]. In colon cancer, CIMP is associated with a poorer prognosis [96]. As for colon cancer, hypermethylation was found associated with a poorer outcome in ACC [50, 51, 93].

Pathophysiological Consequences of Altered DNA Methylation

DNA methylation in gene promoter regions impacts gene expression. Globally, one study reported that methylation could explain the downregulation of ~1/3 of repressed genes in ACC [93]. DNA methylation is thus a major contributor to the transcriptome profile of ACC.

Mechanisms of DNA methylation are not fully clarified. Recently, overexpression of EZH2, a histone methyl transferase, was identified as a potential mechanism explaining CIMP in ACC [97]. DNA methylation can also impact proliferation. Indeed, several studies showed that treatment of ACC NCI-H295R cells with demethylating agents, increased expression of silenced genes, and seemed to decrease cell proliferation, cortisol secretion, and cell invasion [98]. Finally, DNA methylation is linked to histone methylation, and more globally to epigenesis. DNA methylation analyses are probably only the beginning of further epigenetic studies in ACC.

Combining the Omics

Two consortia studies extensively generated a full ACC genomic characterization in two independent international cohorts [50, 51]. Each tumor was studied by transcriptome, miRNome, chromosomal alterations, methylome, and exome sequencing. A global molecular classification has emerged from the combination of omics, which have had highly consistent results between the two cohorts (Fig. 4.7).

Three main molecular groups of ACC are as follows:

- In the first group, a majority of ACC express the poor outcome transcriptome signature, have “noisy” chromosomal alterations, are highly hypermethylated in CpG islands, and present mutations among the few recurrently mutated genes.
- In the second group, ACC present a poor outcome gene expression signature, show a “chromosomal” profile of chromosome alteration, show an intermediate methylation of CpG islands, and also show mutations in the recurrently mutated genes.
- In the third group, ACC present a better outcome gene expression signature, show a “chromosomal” profile of chromosome alteration, no hypermethylation of CpG islands, and no mutation in recurrently mutated genes.

Table 4.6 DNA methylation studies using methylation arrays

Study	Main conclusions of the authors	Genomic technique	Main analytical methods	ACC (N)	Other sample types (N)
Rechache et al. [95]	Methylome discriminates ACC vs. ACA. In their intergenic regions, ACC are globally hypomethylated 52 Hypermethylated and downregulated genes in ACC were identified (including RARRES2, SLC16A9, and GATA6)	Infinium HumanMethylation450 BeadChip (Illumina) ~485,000 individual CpG	Unsupervised classification (hierarchical clustering and principle component analysis)	20	ACA (48), NA (19)
Fonseca et al. [94]	212 CpG islands in the promoter regions were significantly hypermethylated in ACC vs. ACA. For 6 genes selected among these 212 (see later), expression is low in ACC Hypermethylated and down in ACC : CDKN2A, GATA4, DLECl, HDAC10, PYCARD, and SCGB3A1/HINI	Infinium HumanMethylation27k BeadChip (Illumina)	Group comparison	15	ACA (27), NA(6)
Barreau et al. [93] Assié et al. [50]	Focusing on the CpG islands of genes promoter regions, methylome reveals 2 subtypes of ACC, one with hypermethylation, the other without. This hypermethylation is associated with a poor prognosis The transcriptome/methylation correlation showed 1741 genes negatively correlated (including H19, PLAGL-1, GOS2, and NDRG2)	Infinium HumanMethylation27k BeadChip (Illumina)	Unsupervised classification (hierarchical clustering)	51	ACA (84)
Zheng et al. [51]	Unsupervised classification identifies three subtypes of ACC, mainly differentiated by their methylation level in CpG islands of Pgene promoter regions, evoking a CIMP. CIMP is associated with poorer outcome	~27,600 CpG	Survival analysis		
		Infinium HumanMethylation450k BeadChip (Illumina)	Unsupervised classification (hierarchical clustering) survival analysis	79	

ACA adrenocortical adenoma, ACC adrenocortical cancer, *LOH* loss of heterozygosity, *CIMP* CpG island methylator phenotype

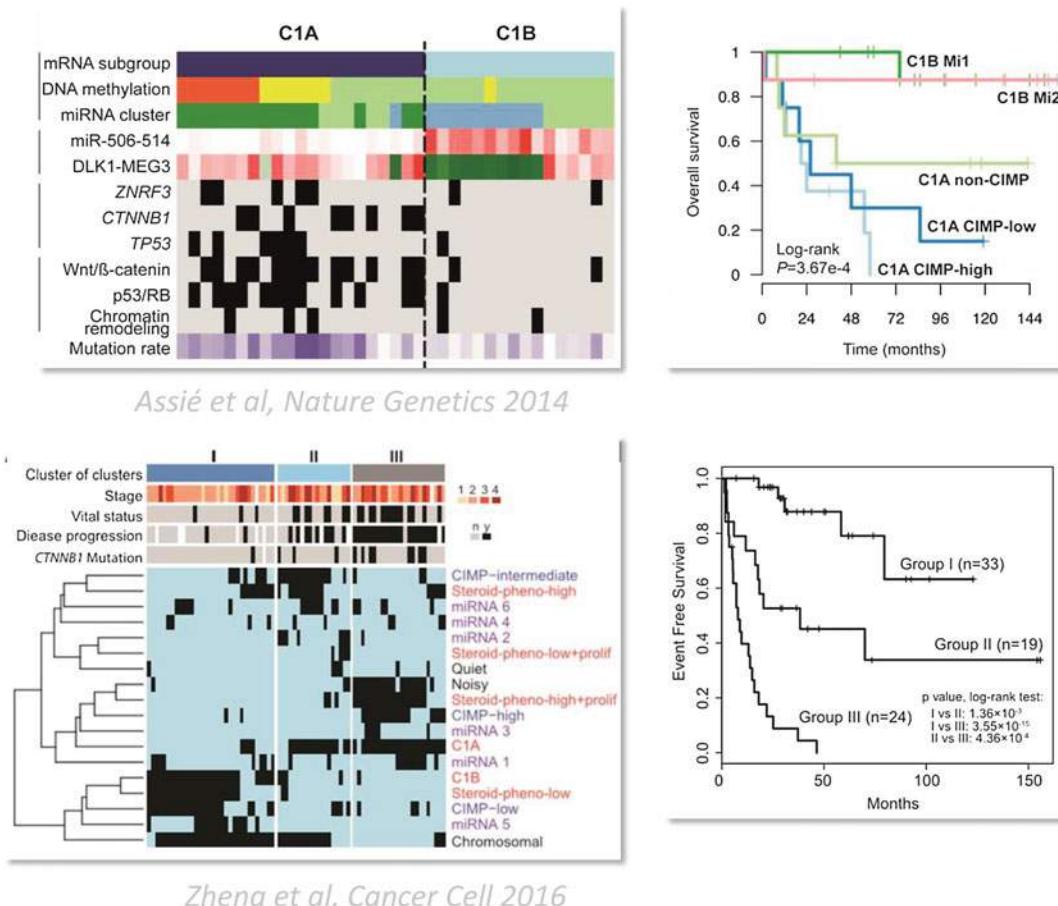


Fig. 4.7 Integrated genomic classifications of adrenocortical carcinomas from European Network for the Study of Adrenal Tumors and The Cancer Genome Atlas cohorts

[50, 51]. Molecular subgroups are associated with different outcome

Interestingly, outcome differs between these three groups: poor for the first group, better for the third group, and intermediate for the second group.

Perspectives

Molecular Characterization of Tumors in Clinical Practice

Molecular characterization of ACC provides information additional to the pathology and immunohistochemistry. Notably, it is now possible to determine a molecular class that is strongly

correlated with prognosis. It is therefore expected that ACC will be systematically typed at the molecular level. Current molecular classifications are based on pan-genomic characterization. Efforts have now begun to try and simplify the global information to targeted molecular features.

Transcriptome

Since transcriptome can properly discriminate poor outcome versus better outcome ACC, one study proposed to try and summarize the total transcriptome to the minimum number of genes [16]. A minimal classifier was proposed, based on the subtraction of 2 genes expression level,

one with high expression in poor prognosis ACC (*BUB1B*), and the other with low expression in poor prognosis ACC (*PINK1*). Thus, poor prognosis would show a high difference, whereas better outcome ACC would show a low difference. This classifier could be validated on two independent cohorts from different centers [16, 99]. This classifier could also demonstrate a prediction independent from extent of disease. A similar approach was adopted to discriminate recurring vs. nonrecurring ACC after complete surgery, using *DLG7-PINK1* [16, 99]. Other differentially expressed genes have been proposed, but require further validation.

Mirnome

Specific miRNAs associated with prognosis have also been proposed. Soon et al. evaluated miR-483-5p and miR-195. They validated on an independent cohort that high miR-483-5p and low miR-195 are associated with poor prognosis [71]. Further studies are needed to confirm these results and potentially confirm that it is indeed the best combination.

Methylome

Methylome studies showed that methylation of CpG islands is strongly associated with prognosis. Barreau et al. proposed to simplify the methylome to targeted measures by MS-MLPA using a commercial kit [93]. A good correlation was reported. However, validation in an independent cohort is required.

What Will Be the Ultimate Targeted Molecular Test?

The ultimate molecular test has to meet the qualities of a prediction test, including being strongly associated with survival, not too expansive, and easy enough to handle. Whether such a test will derive from one single omic or from a combination of omics remains to be determined. Another important question is related to the starting material. Should molecular testing be performed from RNA or from DNA? DNA is more robust than RNA and can potentially be extracted from formalin-fixed and paraffin embedded (FFPE) samples. However, whether DNA-based molecular

prediction is as informative as RNA-based prediction remains an open question. Finally, before proposing the routine use of molecular markers, we have to determine the appropriate place of molecular prediction with regards to nonmolecular predictors.

For diagnosis, considering the high performance of pathology-based malignancy diagnosis in most cases in expert centers, can one expect any benefit from molecular classification? Perhaps in a few instances. In one transcriptome series of adrenocortical tumors, the 2 tumors with a Weiss score of 2, and the 4 tumors with a Weiss score of 3 were all classified as malignant by the transcriptome in the malignancy group [16]. One of these recurred after surgery, but not the other 5 tumors. Does that mean that these 5 tumors could have developed and spread out of the adrenal if not removed? We do not know. However, other molecular features (including IGF2 overexpression and chromosomal alterations) suggest these tumors are carcinomas rather than adenomas.

For prognostication, tumor proliferation index has proven to be a major independent prognostic factor in ACC [100]. Tumor stage, as summarized by the ENSAT staging system, is also an important predictor. It remains to be determined how molecular markers precisely interact with these pathological and clinical parameters in terms of predicting prognosis.

Toward Liquid Biopsies?

Measuring Cell-Free Tumor DNA

In patients with cancer, it is now possible to measure in blood molecular markers reflecting the molecular features of the tumor. The most straightforward application is the detection of cell-free tumor DNA in patients' plasma, by detecting in plasma a specific mutation known to be present in the tumor [101]. In ACC, a set almost 20 genes recurrently mutated has been identified. More than half of the tumors harbor a mutation in these genes. This reduced number of genes may be screened in plasma of ACC patients. Detecting a specific ACC mutation in plasma could have different applications.

Before surgery, it could be a noninvasive way to confirm malignancy. After apparently complete surgery, it could be a way to confirm that no tumor cells remain, provided that sensitivity is sufficient. In addition, in patients with remaining disease it could be a way to monitor the evolution of the tumor burden, providing that quantification methods are reproducible. Finally in theory cell-free tumor DNA is a way to monitor noninvasively the clonal evolution of a tumor, through characterization of new mutations appearing. These new mutations can be the landmark of a genetic evolution of the tumor. However at this stage, clonality of ACC has not been extensively studied, even in tumor samples.

Measuring Circulating miRNA

Chabre et al. recently examined circulating levels of miRNAs [75]. They showed that low miR-195 and high miR-483-5p in the serum of patients ACC are associated with a worse outcome. However, this result was achieved in 12 ACC cases and deserves further validation in independent cohorts [76].

How ACC Genomics Can Impact ACC Treatments

New Therapeutic Targets Identified by Genomics

The global picture provided by ACC genomic studies identified a reduced number of altered signaling pathways. The Wnt/β-catenin pathway activation is one of most common feature of ACC. Molecules modulating this pathway are under evaluation [102]. However, given the ubiquitous role of this pathway, it is not clear whether the antitumor benefits will overcome the likely systemic effects. In the same pathway, *ZNRF3* was identified as a new tumor suppressor gene in ACC. Whether or not *ZNRF3* alterations will be targetable remains an open question. Methylome studies identified a CIMP phenotype in ACC that is associated with a poor prognosis. This opens the question whether demethylating agents such

as decitabine could impact the patients outcome. Several studies showed a lower proliferation of ACC cell-line H295R in vitro with treatment [98].

The recent TCGA paper proposed a list of potentially targetable genes, altered in ACC. The authors based their search on drugs being tested in clinical trials or already approved drugs. They identified 51 potentially targetable alterations in 22/90 ACC [51]. These included cyclin-dependent kinases and proteins involved in DNA repair. Clinical trials will be necessary to confirm these candidates are therapeutic targets for ACC.

Potential Impact of Patient Stratification by Molecular Classification

The global molecular classification identifies different subtypes of ACC. On an individual basis, determining the molecular class of each tumor soon after surgery should help to inform patient treatment. Especially, after a complete surgery, it would probably be recommended to propose an adjuvant therapy for ACC of the molecular class associated with the worse outcome; in contrast, one could propose no adjuvant therapy for ACC of the molecular class associated with the better outcome. Similarly, in cases of patients presenting with metastases or recurrence, it would probably be recommended to propose a systemic chemotherapy for ACC of the molecular class associated with the worse prognosis; in contrast one could propose repeat surgery and/or locoregional therapies for ACC of the molecular class associated with the better outcome. However, before such approaches are translated into clinical practice, such strategies should be validated in clinical trials.

Identifying Predictors of Response to Treatment

A few patients respond to mitotane or to systemic chemotherapy. Although rare, these responses are important. It would be therefore extremely relevant to identify molecular markers predicting these responses. Genomic approaches could help to answer these questions in the future.

Remaining Pathophysiological Questions

Genomic studies have provided an important step forward in understanding ACC pathophysiology. However, many questions remain. First, one-third of ACC do not have any mutation in any recurrent driver genes. What are the drivers for these tumors? How should we look for these alterations? Are there any epigenetic events? In addition, several genomic findings point toward epigenesist, including DNA methylation alterations, and chromatin remodeling genes mutations. Specific studies of ACC epigenesist are probably one of the upcoming challenges in the field. ACC clonality is also an aspect of genomics that remains to be explored. In their methylome study, Rechache et al. could identify a different profile between primary tumors and metastases [95]. It remains an open question how much each omic would vary among the different regions of an ACC. In addition, if considering molecular markers for diagnosis or prognostication, are such markers impacted by the choice of the tumor portion submitted for molecular analysis? Finally one study reported a transcriptome inflammatory signature [51]. Is inflammation impacting ACC tumorigenesis? How? Is there any potential for immunotherapy in ACC, at least in some patients? Many aspects of ACC genomics deserve additional studies.

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Genetics of Pheochromocytoma and Paraganglioma

5

Bruna Babic and Naris Nilubol

Introduction

Pheochromocytomas (PCC) and paragangliomas (PGL) are rare tumors of neural crest origin with an incidence of 2–5 cases per million per year [1]. PCC arise from chromaffin cells of the adrenal medulla, while PGL arise from either sympathetic or parasympathetic paraganglia. PCC and PGL from sympathetic paraganglia are functioning tumors that secrete catecholamines.

These tumors can be of sporadic or hereditary origin. Initial description of the hereditary nature of these tumors cited heritability of about 10 %. To date, approximately 40 % of patients with PCC/PGL have germline mutations in at least one of 14 susceptibility genes [2]. The rate of germline mutation in the pediatric population with PCC/PGL is higher, reaching up to 70 % [3, 4]. This higher rate is attributable to increased recognition resulting from discovery of novel susceptibility genes, awareness of disease inheritance, and better access to genetic testing. The autosomal dominant hereditary syndromes associated with PCC/PGL

include von Hippel–Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN2), neurofibromatosis type 1 (NF1), and hereditary/familial paraganglioma disorders (PGLs 1–4). The genes involved in these syndromes are *VHL*, *RET*, *NF1*, and succinate dehydrogenase (*SDH*), respectively. Other susceptibility genes with germline mutations linked to PCC/PGL include *MAX* and *TMEM127*. Currently, the latter genes do not have specific syndromes associated with mutation.

As the understanding of the genotype–phenotype relationship for these hereditary syndromes has improved, clinical management in patients with PCC/PGL can be tailored specifically to the causal gene(s). This is not only important for diagnosis, but genetic information can be used to identify risk of malignancy or recurrent disease, and to optimize treatments and surveillance for both the patient and family members. In addition, knowledge of the molecular mechanisms that lead to tumor initiation and progression can inform treatment strategy in patients with sporadic and hereditary PCC/PGL.

This chapter summarizes the current body of literature on known PCC/PGL susceptibility genes. Those associated with a hereditary syndrome will be described first, followed by a description of recently discovered germline mutations that as of yet have not been associated with a specific hereditary syndrome. The management of individuals with PCC/PGL based on the precise germline mutation is discussed.

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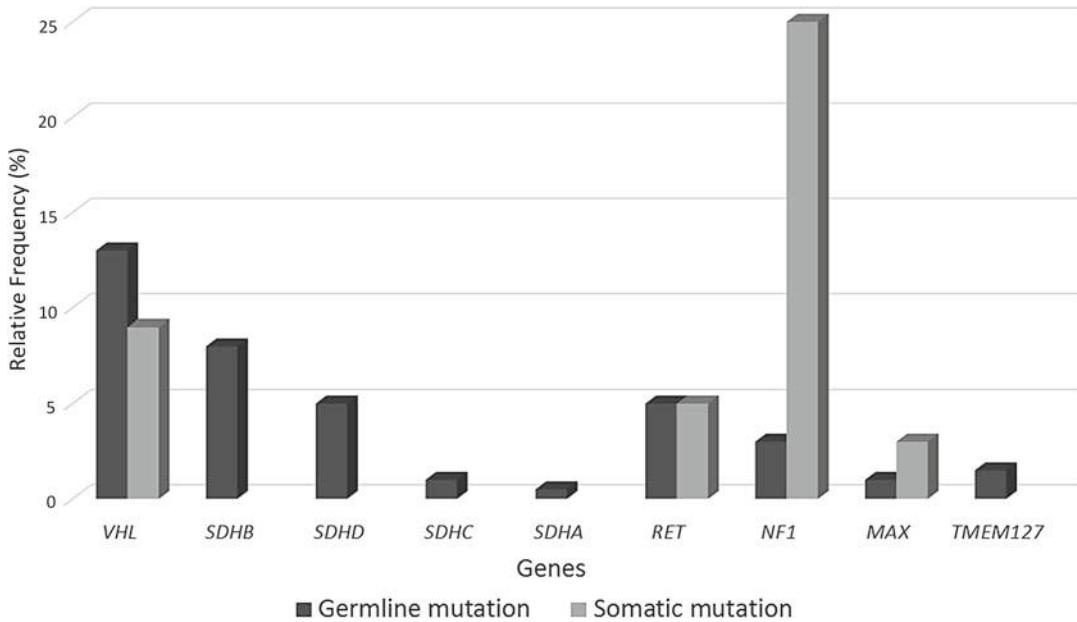


Fig. 5.1 Relative frequency of germline and somatic mutations of specific genes in pheochromocytomas and paragangliomas. Germline and somatic mutations are shown in dark gray and light gray, respectively. *VHL* von Hippel–Lindau; *SDH* succinate dehydrogenase; *NF1* neurofibromin 1; *MAX* MYC-associated factor X; *TMEM127* transmembrane protein 127. (Adapted from Daha [2] with permission)

Germline Susceptibility Genes in PCC/PGL

The frequency of germline mutations in PCC/PGL is summarized in Fig. 5.1 [2]. The susceptibility genes associated with hereditary PCC/PGL are categorized into two groups based on gene expression profile and the dysregulated pathways associated with driver mutations [2].

Cluster 1 or pseudo-hypoxia cluster: Genes in this cluster are involved in oxygen-independent stabilization of hypoxic-inducible factor (HIF) and activation of the downstream oncogenic signaling pathways. The susceptibility genes in this cluster include posttranslational regulators of HIF stability such as *VHL* and, in rare cases, propyl hydroxylase (*PHD2* or *EGLN1*) [5, 6]. In addition, mutation of genes involved in the Krebs cycle, such as the four subunits of *SDH* (*SDHA-SDHD*), SDH assembly factor (*SDHAF2*), and fumarate hydratase

(*FH*), causes accumulation of intracellular metabolites that inhibit the function of *PHD2* [7–12]. These mutations result in a pseudo-hypoxic effect, leading to aberrant accumulation of the HIF transcription factor [2]. HIF is composed of two subunits, HIF1A and HIF2A. HIF1A interacts with HIF2A via dimerization and acts as a transcription factor that allows adaptation to hypoxia (Fig. 5.2). When this mechanism is lost due to mutation, angiogenesis occurs [13].

Cluster 2 or kinase signaling cluster: This cluster includes genes associated with tyrosine kinase receptor and activation of downstream signaling activity. These genes include *RET*, neurofibromin 1 (*NF1*), transmembrane protein 127 (*TMEM127*), MYC-associated factor X (*MAX*), and *KIF1B β* [14–18]. Activation of tyrosine kinase receptors and downstream signaling pathways, such as PI3K-AKT-mTOR and MYC, affects glycolysis and synthesis of proteins, nucleic acids, amino acids, and fatty acids and promotes cell growth (see Fig. 5.2) [2].

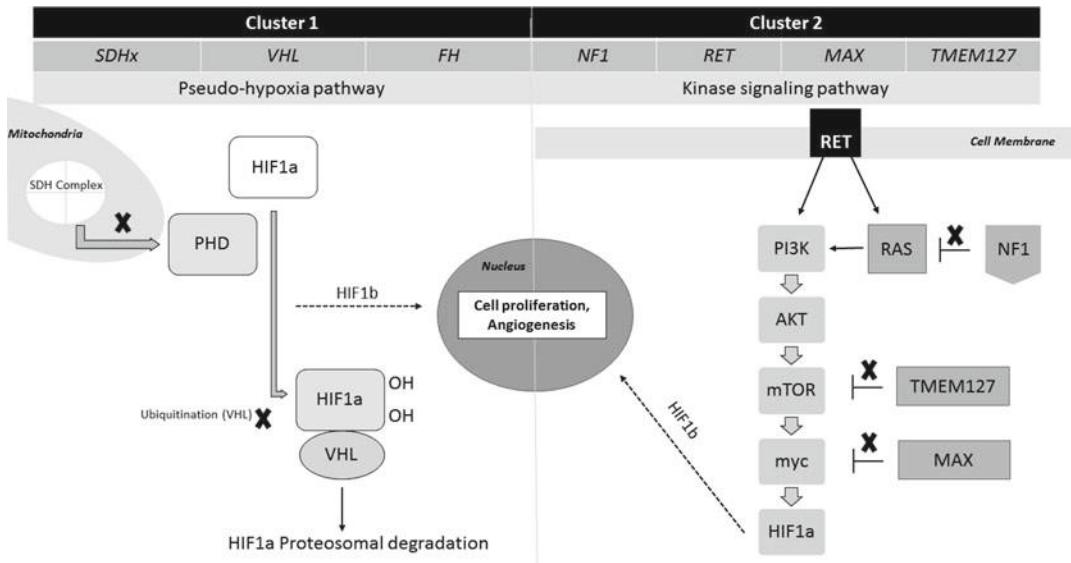


Fig. 5.2 Two groups of susceptibility genes and signaling pathways associated with pheochromocytomas and paragangliomas. *Solid arrows*: normal pathway. *Dashed arrows*: pathway in hypoxia and/or with gene mutations. *Solid cross*: presence of mutation affecting pathway

SDH-Related Mutations and Familial Paraganglioma Syndromes

Familial paraganglioma syndromes are caused by mutations in the genes that encode for the sub-units of the SDH complex, also known as mitochondrial complex II. Complex II is part of the Krebs cycle and the electron transport system within the mitochondria. The function of the electron transport system is to produce ATP via an electrochemical gradient in the membrane of the mitochondria. Complex II catalyzes the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol. The core of SDH complex is made up of two subunits: a flavoprotein (*SDHA*) and an iron-sulfur protein (*SDHB*). The core is anchored to the inner mitochondrial membrane by the other two subunits (*SDHC* and *SDHD*). The SDH complex catalyzes oxidation of succinate to fumarate. In turn, electrons are transferred to ubiquinone in the electron transport chain (Fig. 5.3).

SDH mutations result in defective SDH proteins with shorter half-lives. Insufficient SDH complex causes pseudo-hypoxia as succinate accumulates in the mitochondria. Excessive suc-

cinate is exported to the cytosol where it stabilizes HIF1A and decreases degradation by inhibiting PHD-mediated hydroxylation of HIF1A. Stabilized HIF1A translocates into the nucleus, forming heterodimeric HIF, and inducing a pseudo-hypoxic response [19].

Familial paraganglioma syndromes have distinct clinical phenotypes based on the specific SDH mutation. The first gene to be identified was *SDHD*, followed by *SDHC*, *SDHB*, and *SDHA* [7, 9–12]. Recently, SDH-related genes have been discovered, including *SDHAF1* and *SDHAF2* that encode cofactors [20]. Because of the rarity, clinical information from patients with mutations in *SDHA*, *SDHC*, and *SDHAF2* is limited. *SDH* mutations are frequently discovered without prior family history, perhaps due to a low rate of penetrance. Furthermore, maternal imprinting has been found in patients with *SDHD* and *SDHAF2*, an epigenetic modification that may obscure familial inheritance [21]. *SDH* mutations cause PCC and extra-adrenal PGL, including head and neck PGL. Additional tumors associated with *SDH* mutations include renal cell carcinoma and gastrointestinal stromal tumors. Renal cell carcinoma has been reported in

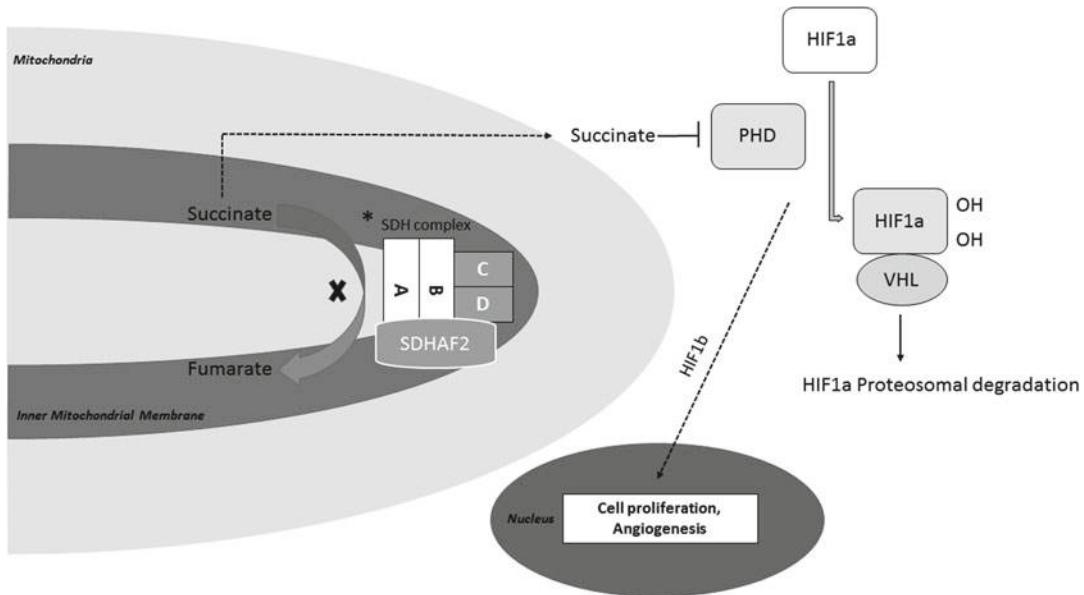


Fig. 5.3 Pathogenesis of familial paraganglioma syndromes is caused by mutations in the *SDH* gene that form the SDH complex, also known as mitochondrial complex II (*). Excessive succinate results in pseudo-hypoxic

response. Solid arrows: normal pathway. Dashed arrows: pathway in hypoxia and/or with gene mutations. Solid cross: presence of mutation affecting pathway

patients with *SDHB*, *SDHC*, and *SDHD* mutations. It is estimated that 14% of *SDHB* carriers develop renal cell carcinoma [22–24]. The Carney–Stratakis dyad consists of PCC/PGL and gastrointestinal stromal tumors, while the Carney triad includes additional pulmonary chondromas [25, 26]. Germline *SDHB*, *SDHC*, and *SDHD* mutations have been reported in patients with Carney–Stratakis dyad; however, the genetic defect in patients with Carney triad remains unknown [27]. Pituitary adenoma and neuroblastoma have also been reported in patients with *SDH* mutations [28, 29]. Clinical characteristics of patients with germline *SDH* mutations are summarized in Table 5.1 [30].

Succinate Dehydrogenase Subunit D

SDHD mutation was discovered in 2000 in patients with hereditary PGL of the head and neck, predominantly at the carotid body. *SDHD* mutation causes familial paraganglioma 1 (PGL1) syndrome. The gene is located on chromosome 11q23 and encodes for a protein that is present in a hydrophobic component of complex II [10]. The *SDHD* protein is a subunit of cyto-

chrome b that functions along with SDHC to anchor subunits A and B to the inner mitochondrial membrane. Specifically, SDHC interacts with SDHB and attaches mitochondrial complex II to the inner mitochondrial membrane. Inheritance of these mutations is predominantly paternal [31], although recently maternal transmission has been described [32].

The tumors in patients with *SDHD* germline mutations predominantly are nonfunctioning head and neck PGL. However, subsequent studies found that patients infrequently present with PCC and extra-adrenal PGL of the abdomen [33]. The risk of malignancy of PCC/PGL with *SDHD* mutations is low.

Succinate Dehydrogenase Subunit C

The discovery of *SDHD* led to identification of other *SDH* subunits. *SDHC* was discovered shortly after *SDHD*; mutations are associated with familial paraganglioma syndrome 3 (PGL3). Of all the *SDH* mutations, *SDHC* mutation is the least common germline mutation. *SDHC* mutations are most commonly associated clinically with head and neck PGLs. Extra-adrenal PGLs

Table 5.1 Characteristics of hereditary diseases associated with pheochromocytomas and paragangliomas

Disease	Gene	Protein function	Chromosomal location	Phenotypic features	Focality (single vs. multiple)
von Hippel–Lindau	<i>VHL</i>	E3 ubiquitin ligase	3p25.3	Central nervous system and/or retinal hemangioblastoma, renal cell carcinoma, pheochromocytoma, paraganglioma, pancreatic neuroendocrine tumors and cysts, endolymphatic sac tumors, papillary cystadenoma of the epididymis and broad ligament	Multiple
Multiple endocrine neoplasia type 2	<i>RET</i>	Receptor tyrosine kinase	10q11.21	(2A) Medullary thyroid cancer, hyperparathyroidism, pheochromocytoma (2B) Medullary thyroid cancer, marfanoid habitus, mucocutaneous neuromas, gastrointestinal ganglioneuroma, pheochromocytoma	Multiple
Neurofibromatosis type 1	<i>NFI</i>	GTPase-activating protein	17q11.2	Café-au-lait spots, axillary and inguinal freckling, Lisch nodules, neurofibroma, osseous lesions, optic gliomas, pheochromocytoma	Single
Familial hereditary paraganglioma syndrome	<i>SDHx</i>	Succinate dehydrogenase/ mitochondrial complex		Pheochromocytoma, paraganglioma	
– PGL 1	<i>SDHD</i>	Anchoring subunit	11q23.1	Head and neck paraganglioma with rare extra-adrenal abdominal paraganglioma or pheochromocytoma	Multiple
– PGL 2	<i>SDHA/F2</i>	Assembly factor	11q12.2	Head and neck paraganglioma	Multiple
– PGL 3	<i>SDHC</i>	Anchoring subunit	1q23.3	Head and neck paraganglioma with rare extra-adrenal abdominal paraganglioma or pheochromocytoma	Multiple
– PGL 4	<i>SDHB</i>	Catalytic subunit	1p36.13	Extra-adrenal paraganglioma, rarely pheochromocytoma or head and neck paraganglioma	Multiple
– PGL 5	<i>SDHA</i>	Catalytic subunit	5p15.33	Pheochromocytoma or extra-adrenal paraganglioma	Single
Hereditary pheochromocytoma	<i>MAX</i>	Transcription factor	14q23.3	Pheochromocytoma	Single
Hereditary pheochromocytoma	<i>TMEM127</i>	Transmembrane protein involved with mTOR	2q11.2	Pheochromocytoma	Single

Adapted from Dahia [2], and from Favier et al. [30], with permission
SDHx succinate dehydrogenase mutation, *mTOR* mechanistic target of rapamycin, *PGL* paraganglioma

have been reported [11, 34]. The gene is located on chromosome 1q21 and encodes a large sub-unit of cytochrome b (cybL) in the SDH complex [11]. *SDHC* does not have a parental origin of transmission like *SDHD*. The tumors are mostly benign and seldom multifocal [35].

Succinate Dehydrogenase Subunit B

Following *SDHD*, *SDHB* mutations were discovered. *SDHB* is the most commonly mutated sub-unit of SDH and is frequently associated with abdominal and extra-adrenal PGL [2]. *SDHB* is located on chromosome 1q36. The *SDHB* protein is composed of three iron-sulfur clusters, creating a hydrophilic domain. It binds with the *SDHA* protein to create a core, which then binds to *SDHC* and *SDHD* to anchor the core to the inner mitochondrial membrane. The clinical phenotype of patients with *SDHB* mutations is primarily extra-adrenal PGL. Patients with *SDHB* germline mutations commonly present with multiple synchronous or metachronous tumors at a younger age. There is a higher risk of metastatic disease and a poor prognosis associated with *SDHB* mutations [36–38]. However, *SDHB* mutations have lower penetrance compared to other PGL syndromes with PCC/PGL, occurring in 30 % by 80 years of age to 45 % by 40 years of age [23, 36, 39].

Succinate Dehydrogenase Subunit A

SDHA is located on chromosome 5p15.33 and encodes a large hydrophilic protein subunit of the SDH complex. However, for many years after its discovery, no known germline mutations were identified and associated with a clinical hereditary presentation of PCC or PGL. Mutation in *SDHA* is associated with Leigh syndrome, which was first described in 1951 and is also referred to as juvenile subacute necrotizing encephalomyopathy. Unlike *SDH*-related PGL, Leigh syndrome exhibits recessive inheritance requiring mutation in both *SDHA* alleles. It is an inherited neurometabolic disorder that affects the central nervous system and leads to progressive muscular weakness. It was not until 2011 that Burnichon et al. discovered a germline mutation in a patient with an extra-adrenal PGL [40]. Evaluation of a

larger cohort indicated that *SDHA* mutations accounted for 4.5 % of germline mutations of PGL and PCC [40]. PCC, head and neck PGL, extra-adrenal PGL, and pituitary adenoma have been reported in patients with *SDHA* germline mutations [40–42].

SDHAF2

SDHAF2 is an SDH-related gene residing on chromosome 11q12.1. The encoded protein functions as an assembly protein for *SDHA*, a flavoprotein that requires *SDHAF2* for flavination and thereby activation. Mutations in *SDHAF2* cause *SDHA* to be inactive by loss of flavination. Individuals who harbor mutation in *SDHAF2* develop head and neck PGL [12], and mutations have been associated with at least 15 cases of PGL arising from parasympathetic ganglia. The penetrance is 100 % by the age of 45 years [43, 44].

SDHAF1

SDHAF1 (succinate dehydrogenase complex assembly factor 1) is a protein involved in the assembly of SDH complex. It currently does not have a direct link to the development of PCC/PGL. Mutations in the gene are associated with mitochondrial complex deficiency and were described in 2009 as being involved in the development of infantile leukoencephalopathy [45].

von Hippel–Lindau disease

VHL mutations cause von Hippel–Lindau disease, an autosomal dominant familial cancer syndrome. Primary manifestations of the disease include retinal or central nervous system hemangioblastomas and clear cell renal cell cancer. Other tumors include PCC, pancreatic neuroendocrine tumors, and endolymphatic sac tumors [46] and very rarely PGL of the head and neck [47]. Individuals presenting with one of these tumors and a family history are diagnosed with *VHL*. In those with no family history, at least two hemangioblastomas or presence of hemangioblastoma and one of the other types of tumors is required for diagnosis; in these individuals, there

Table 5.2 Clinical characteristics of different von Hippel–Lindau disease subtypes (1, 2A, 2B, 2C)

VHL disease subtype	Mutation type	Phenotype
1	Deletion, nonsense, frame shift	Clear cell renal cell cancer (ccRCC), hemangioblastoma
2a	Missense	Hemangioblastoma, pheochromocytoma
2b	Missense	ccRCC, hemangioblastoma, pheochromocytoma
2c	Missense	Pheochromocytoma

Each is characterized by degree of penetrance of each type of tumor. von Hippel–Lindau (VHL) disease subtype, type of mutation, and phenotype expression

ccRCC clear cell renal cell cancer

is a high likelihood of a *VHL* de novo germline mutation [48].

VHL disease has different clinical subtypes (1, 2A, 2B, 2C), each characterized by the degree of penetrance of each tumor type. Table 5.2 summarizes the tumors associated with each clinical subtype. Extra-adrenal PGL have been described in VHL [47, 49]; however, the predominant tumors are PCC [48]. Clinical subtype 1 of VHL disease never has PCC, in comparison to clinical subtype 2 in which individuals develop PCC. Clinical subtype 2C exclusively presents with PCC, while 2A and 2B are differentiated based on the risk of developing clear cell renal cancer, with 2A having low risk in comparison to the high-risk 2B subtype [50]. Patients with VHL disease typically develop norepinephrine or normetanephrine-producing PCC, with a high rate of synchronous or metachronous bilateral PC. The age of onset of PCC/PGL in patients with VHL disease is approximately 30 years. Although malignant PCC/PGL in patients with VHL syndrome is rare, recurrent and multifocal PCC can occur.

Germline mutation of *VHL* was first described in 1993, and its location was mapped to chromosome 3p25.3. The gene spans three exons and encodes for two proteins [51], both of which have tumor suppressor activity. The proteins encoded by the *VHL* gene form a complex that is involved in protein degradation. The *VHL* protein complex

causes polyubiquitylation of its substrates that marks them for proteolytic degradation. Two of the polyubiquitylated substrates are HIF1A and HIF2A. A decrease of HIF1 α and HIF2 α degradation in *VHL*-mutant tumors leads to HIF accumulation and subsequent induction of multiple downstream targets [50]. HIF2A has more oncogenic properties as it promotes the activity of the oncogenic MYC protein. Most *VHL* mutations preferentially impair degradation of the HIF2A subunit compared with HIF1A in vitro [52], and HIF2A is preferentially upregulated in *VHL*-mutant PCC/PGL [53–55]. The role of HIF1A in PCC/PGL remains unclear. Unlike renal cell carcinoma, no mutations or deletions in *HIF1A* have been reported in sporadic PCC/PGL [56]. The function of HIF1A in cancer may be tissue dependent or may be epigenetically regulated. Unlike renal carcinoma-related *VHL* mutations that involve deletions and truncations that severely destabilize *VHL* function, *VHL* mutations associated with PCC/PGL are predominantly missense [50].

Although the role of HIF in pathogenesis of VHL-associated tumors is clear, mutant *VHL* can be tumorigenic independently of HIF activation [57]. HIF-unrelated *VHL* mutations were initially identified in patients with VHL type 2C who develop only PCC without other VHL-related manifestations [58].

Multiple Endocrine Neoplasia Type 2

Multiple endocrine neoplasia type 2 (MEN2) is a rare autosomal dominant hereditary syndrome caused by germline activating mutations of the *RET* proto-oncogene. The prevalence of these mutations varies between 1 per 30,000 and 1 per 50,000 individuals [59–62]. Because *RET* encodes a transmembrane tyrosine kinase receptor, the study of *RET* provides insights into the oncogenic effect of aberrant activation of tyrosine kinase signaling and the development of multiple kinase inhibitors currently used clinically. In addition, knowledge of the genotype–phenotype association derived from patients with various *RET* mutations enables tailored disease

Table 5.3 Genotype–phenotype associations of *RET* protooncogene and risk of developing pheochromocytoma, medullary thyroid cancer, and other phenotypes

<i>RET</i> mutation	Incidence of pheochromocytoma (%)	Medullary thyroid cancer risk	Incidence of hyperparathyroidism (%)	Presence of CLA/HD
533	10	+	–	N/N
609	10–30	+	10	N/Y
611	10–30	+	10	N/Y
618	10–30	+	10	N/Y
620	10–30	+	10	N/Y
630	10–30	+	10	N/N
631	~50	+	–	N/N
634	~50	++	10–30	Y/N
666	10	+	–	N/N
768	–	+	–	N/N
790	10	+	–	N/N
V804L	10	+	10	N/N
V804M	10	+	10	Y/N
883	~50	++	–	N/N
891	10	+	10	N/N
912	–	+	–	N/N
918	~50	+++	–	N/N

Adapted from Wells et al. [63]

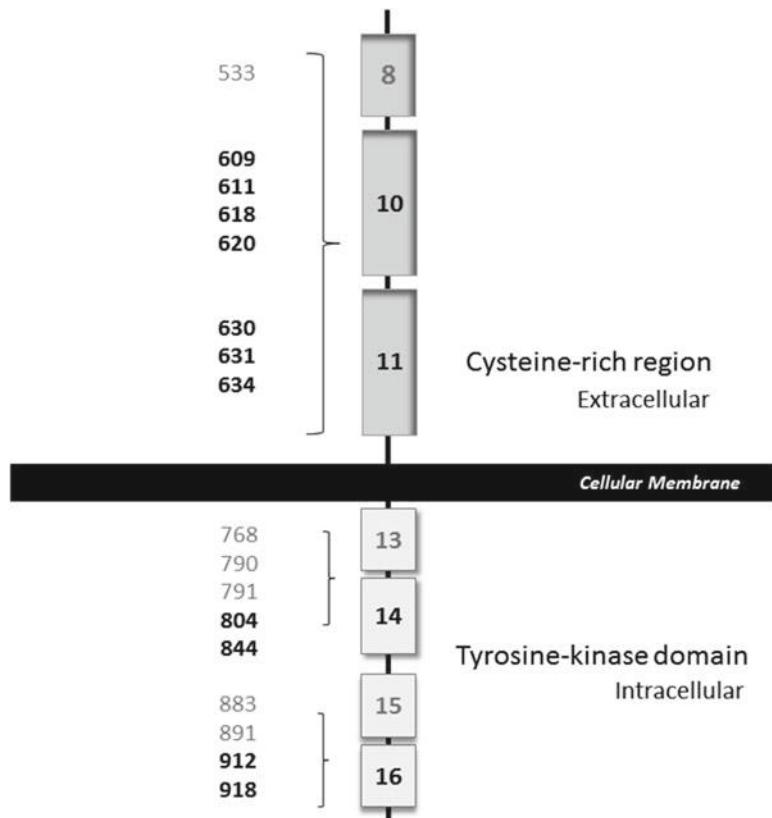
CLA cutaneous lichen amyloidosis, HD Hirschsprung disease, + = moderate risk, ++ = high risk, +++ = highest risk, Y = present, N = not present

management based on patients' risk of developing medullary thyroid cancer, PCC, and other phenotypes (Table 5.3) [63].

Mutations in the *RET* gene were first linked to MEN2 in 1993 [64]. *RET* is located on chromosome 10q11.2 and is composed of 21 exons. The encoded tyrosine kinase receptor comprises an extracellular domain containing a ligand-binding site, a cysteine-rich region (exons 10 and 11), a transmembrane domain, an intracellular part with two tyrosine kinase domains (exons 13–15), and an intracellular catalytic core (exon 16) (Fig. 5.4) [60, 65]. *RET* is expressed in neural and neuroendocrine cell lineages such as thyroid C-cells, adrenomedullary chromaffin cells, and parathyroid cells [66]. *RET* is essential for neural crest cell migration; the development of enteric nervous system; and for the proliferation, differentiation, and survival of these cell types once they reach their destinations [67]. *RET* is the receptor for a family of soluble neurotrophic factor ligands, the glial cell line-derived neurotrophic factors (GDNFs). Upon binding, *RET* dimeriza-

tion and autophosphorylation of intracellular tyrosine residues recruits adaptor and signaling proteins to stimulate multiple downstream pathways such as PI3K/AKT/mTOR and RAS/MAPK/ERK [67]. Thus, constitutive activation of *RET* causes hyperplasia of thyroid C-cells, adrenal medulla, and parathyroid gland. Alteration in the intracellular catalytic core (mutations in codon 918, classical MEN2B genotype) has the highest transforming capacity; mutations that disrupt ligand-independent dimerization and cross-phosphorylation (mutations in codon 609, 611, 618, 630, and 634) have intermediate activity; and mutations that interfere with ATP binding (mutations in codons 768, 790, 791, 804, and 891) have the lowest transforming activity [65, 68]. This results in variable risks of developing medullary thyroid cancer and distinct clinical manifestations of MEN2. MEN2A-type *RET* mutations occur in the extracellular domain of *RET* and cause ligand-independent homodimerization and aberrant activation of PI3K–AKT, RAS, p38 MAPK, and JUN N-terminal kinase

Fig. 5.4 The RET tyrosine kinase receptor comprises an extracellular domain containing a ligand-binding site, a cysteine-rich region (exons 10 and 11); a transmembrane domain, an intracellular part with two tyrosine kinase domains (exons 13–15), and an intracellular catalytic core (exon 16)



pathways, resulting in the stimulation of cell growth, differentiation, and survival [69, 70]. Patients with MEN2A, in addition to PCC, develop bilateral medullary thyroid cancer and hyperparathyroidism secondary to parathyroid hyperplasia. MEN2B-causing mutations, by contrast, are in the few codons that affect the catalytic site of the kinase and lead to the loss of substrate specificity [70]. MEN2B, in addition to PCC and bilateral medullary thyroid cancer, is characterized by a spectrum of different manifestations, including mucosal neuroma, intestinal ganglioneuromas, and skeletal abnormalities (e.g., marfanoid habitus) (Fig. 5.5) [71]. *RET* mutation at codon 634 is by far the most common, accounting for 85 % of MEN2A cases. The penetrance of PCC in patients with *RET* codon 634 mutations is 20 % of individuals by age 20, and 67 % by the age of 50. In the same cohort, 21 % of individuals presented with PCC prior to diagnosis of medullary thyroid cancer [72]. The significance of this observation is that despite the

more commonly assumed presentation of thyroid nodules or diagnosis of medullary thyroid cancer, those that present with PCC alone should be considered for genetic screening for *RET* mutation. While de novo *RET* germline mutations are common (95 %) in patients with MEN2B, de novo *RET* germline mutations occur in 4–10 % of patients with MEN2A [73]. Individuals with MEN2 commonly develop bilateral PCC [74]. However, the risk of malignancy is very low. Thus, partial adrenalectomy to preserve adrenal cortical function is recommended in select cases.

Neurofibromatosis Type 1

There are two types of neurofibromatosis, neurofibromatosis 1 and neurofibromatosis 2 (NF1 and NF2, respectively). NF1 is a relatively common autosomal dominant inherited syndrome affecting about 1 in 3,000 individuals [75]. NF1 was previously known as von Recklinghausen's

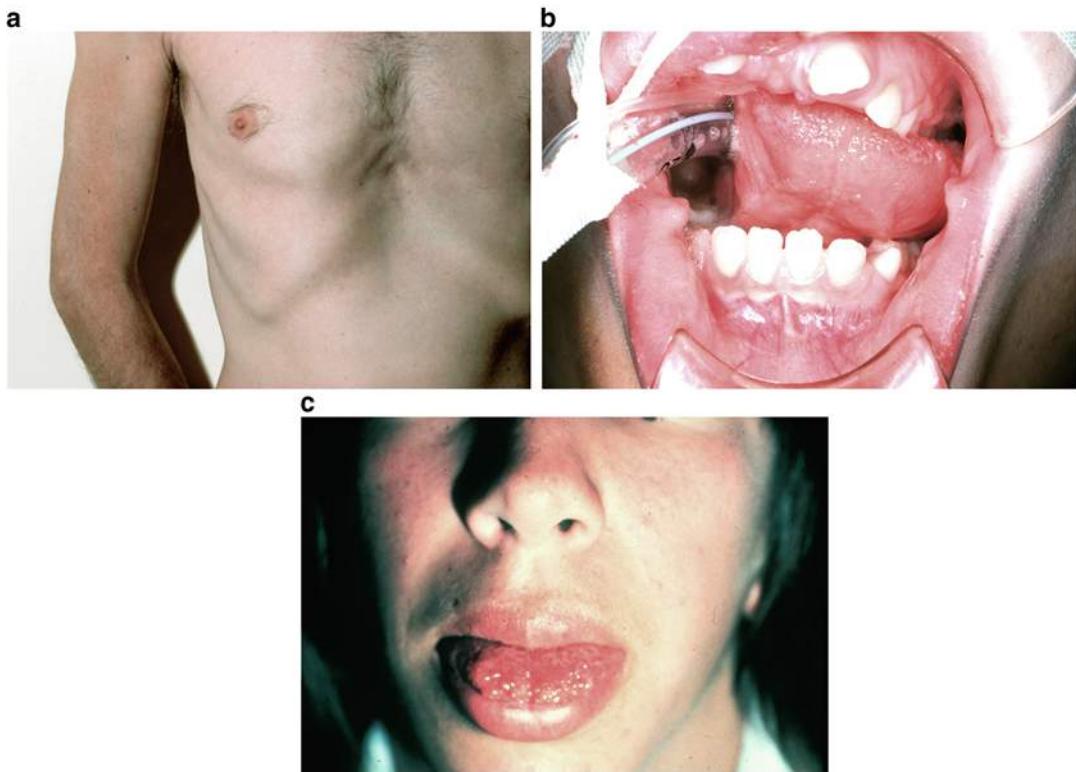


Fig. 5.5 MEN2B physical exam features including marfanoid body habitus with pectus excavatum (a), poor dentition (b) and mucosal neuromas (c). (From Kebabew et al. [71] with permission)

neurofibromatosis or peripheral neurofibromatosis and represents the majority of neurofibromatosis cases (90%). Patients with NF1 are at risk of developing benign and malignant tumors of the central and peripheral nervous system as well as other malignant tumors. Tumors associated with NF1 include glioma of the optic pathway, glioblastoma, malignant nerve sheath tumor, gastrointestinal stromal tumor, breast cancer, leukemia, PCC, duodenal carcinoid tumor, and rhabdomyosarcoma [76]. NF2 is an autosomal dominant disorder caused by a different genetic mutation (merlin) that affects about 1 in 50,000 individuals. NF2 is characterized by acoustic and central neurofibromas and is not associated with PCC.

NF1 is diagnosed based on clinical presentation, which guides genetic testing. *NF1* gene testing is usually reserved for uncommon presentations or reproductive decision-making [76]. A National Institutes of Health consensus

from 1987 outlines the diagnostic criteria for clinical diagnosis of NF1 [77]. NF1 is diagnosed if an individual has two or more of the following presentations: six or more café-au-lait macules, two or more neurofibromas, axillary or inguinal region freckling, optic glioma, two or more iris hamartomas (Lisch nodules), an osseous lesion or thinning of long bones, or a first-degree relative with diagnosis of NF1 [78]. PCC occur in less than 1% of individuals with NF1 and are predominantly unilateral (84%) [79]. The age of onset is typically in the 4th decade of life, similar to those with sporadic disease [80].

NF1 is caused by mutations in the *NF1* gene, a gene located on chromosome 17q11.2 that is composed of 60 exons and encodes a 220-kDa cytoplasmic GTPase activating protein called neurofibromin [81]. Neurofibromin functions as a negative regulator of the *RAS* proto-oncogene. Neurofibromin converts guanosine triphosphate bound Ras to an inactive guanosine

diphosphate bound configuration. When neurofibromin expression is decreased or lost secondary to mutation, the unhindered presence of active Ras leads to increased cell growth thereby promoting tumor formation [82]. mTOR is an important downstream signaling molecule that is aberrantly activated in *NF1*-deficient malignant peripheral nerve sheath tumors and in PCC/PGL [83, 84].

Currently, there are no specific guidelines for surveillance of patients with *NF1* for PCC. Previously it was believed that when presenting with hypertension, the individual should be screened for PCC since studies have shown that such individuals have a 20–56% chance of having a PCC [75]. However, a small cohort of *NF1* patients were all diagnosed with PCC incidentally, and only one of six *NF1* patients had hypertension. This suggests that patients with *NF1* should be screened for PCC prior to development of hypertension [85].

Pheochromocytomas Associated With *TMEM127* Mutations

TMEM127 encodes a transmembrane protein involved in regulating the mTOR signaling pathway. Qin et al. [16] initially described *TMEM127* in 2010 as a transmembrane-encoding gene on chromosome 2q11.2 which was identified from a cohort of 103 cases without previously defined genetic mutation who predominantly had PCC. Prior to this, the *TMEM127* gene was referred to as the FP gene for familial PCC, stemming from its identification via linkage analysis and characterization as a tumor suppressor gene with loss-of-function mutations. In the study by Qin et al., truncating *TMEM127* germline mutations were found in 30% of patients with familial PCC, and 3% of patients with “sporadic” PCC had germline *TMEM127* mutations [16]. PCC associated with *TMEM127* mutations have a transcriptional profile similar to that of PCC associated with *RET* and *NF1* mutations [16, 86]. *TMEM127* is believed to be involved in the mTOR pathway; human *TMEM127*-mutant PCC and cell lines depleted of *TMEM127* have increased mTOR signaling [87, 88]. The loss of

TMEM127 disrupts the early-to-late endosomal transition and enhances lysosomal biogenesis which affects mTOR distribution [88]. The exact function of *TMEM127* and the mechanisms by which it affects mTOR signaling are not fully understood. Thus far, about 30 *TMEM127* germline mutations have been identified [2]. These mutations are insertions or deletions, nonsense or splice site mutations that lead to truncated *TMEM127* protein [16, 86].

Although no specific hereditary syndrome is associated with *TMEM127* mutations, initial studies have made several observations about the characteristics of individuals harboring mutations. The penetrance of germline *TMEM127* is low; only 20% of patients carrying *TMEM127* germline mutations report a family history of PCC [89]. *TMEM127* mutations were predominantly found in individuals with PCC [89, 90], although subsequent studies showed that *TMEM127*-mutation carriers may present with extra-adrenal PGL, including head and neck PGL [91]. Bilateral PCC are common [16, 86], but the majority are unilateral with a similar mean age of presentation as those with sporadic tumors (42.8 years and 43.2 years, respectively). The risk of malignant PCC/PGL in *TMEM127* mutation carriers is low [89].

Pheochromocytomas Associated With *MAX* Mutations

The association between MYC associated factor X (*MAX*) gene and hereditary PCC was first described in 2011 [17]. *MAX* is located on chromosome 14 and encodes a transcription factor that belongs to the basic helix-loop-helix leucine zipper (bHLHZ) family of proteins. Similar to *TMEM127* mutations, mutations in *MAX* have not been linked to a specific hereditary syndrome. Commonly, mutations in *MAX* result in an early stop codon, which causes exon skipping. These mutations affect the ability of *MAX* to appropriately interact with *MYC* within the *MYC/MAX/MXDI* complex; *MYC* is an oncogene involved in many human cancers. *MAX* can form complexes with other bHLHZ proteins

that oppose MYC-mediated activation and inhibit cell growth and promotes cell differentiation [92]. In vivo studies using PCC xenografts show that the reintroduction of MAX in MAX-depleted tumors results in growth arrest, supporting the repressive function of MAX in PCC [92, 93]. In its original description, a paternal mode of transmission was proposed as the mutated allele was paternal in origin in the majority of cases. In this original group, three of eight first-affected family members had metastatic disease, suggesting *MAX* mutations are associated with more aggressive disease [17]. Overall, the rate of germline mutation is low (1.12 %), and it has been predominantly found in individuals with bilateral PCC or multiple PCC in one adrenal gland (68.4 %) [94].

Other Diseases Associated with Pheochromocytomas and Paragangliomas

Other syndromes have been described that are associated with PGL. Some do have a known mutation while others do not.

Carney triad was described in 1977. It is a triad that manifests with gastric stromal tumors (GIST), pulmonary chondroma, and abdominal PGL. The presence of at least two of the tumor types is needed for diagnosis. Other tumor types, including esophageal leiomyomas and adrenocortical tumors, have also been described [95] some of which may be functioning [96].

Females have a higher prevalence of Carney triad, and the first tumor often identified is a GIST in young patients. It has been recommended that young patients diagnosed with GIST have regular follow up to screen for development of the other tumor types [97]. To date, there is no known causative mutation for Carney triad. Recent studies evaluated the role of SDH complex mutation; although no association was identified, it is believed that Carney triad is caused by alterations in the mitochondrial complex [98].

Carney–Stratakis syndrome manifests with GIST and extra-adrenal PGL [99]. The GIST described as part of this dyad were once classi-

fied as wild-type GISTs, lacking the common *KIT* and *PDGFRA* mutations. Germline *SDHB*, *SDHC*, and *SDHD* have been reported in patients with Carney–Stratakis dyad [27]. The GISTs in this dyad have SDHx deficiency [100].

Biochemical Profiles in Patients with Hereditary PCC/PGL

Plasma free-metanephines and/or urine fractionated metanephines are routinely used to screen for PCC/PGL as they are highly sensitive and specific for PCC/PGL. In addition to clinical presentations, biochemical profile can help select appropriate genetic testing. Because those with VHL syndrome have low expression of phenylethanolamine-N-methyltransferase (PNMT), patients commonly have elevated norepinephrine and normetanephrine but not epinephrine and normetanephrine [101, 102]. On the contrary, MEN2-associated PCC frequently over-express *PNMT*, and patients present with predominantly elevated epinephrine [102]. Patients with *NF1* germline mutations have elevated norepinephrines and normetanephrines. Patients with *SDHx* mutations have a normetanephrine predominant biochemical profile. In addition, patients with *SDHx* germline mutations can have high levels of dopamine and methoxytyramine [102]. The head and neck PGL usually are biologically inactive. The biochemical profile of PCC/PGL associated with *TMEM127*, *SDHA*, and *MAX* mutation has not been well documented.

Somatic Gene Mutations in PCC/ PGL

Sporadic PCC/PGL is defined as a tumor that occurs in individuals without a family history and who do not have a germline mutation in known susceptibility genes. Somatic mutations of *NF1*, *VHL*, *RET*, *HIF2A*, *HRAS*, *BRAF*, *TP53*, and *MAX* can be detected in sporadic PCC/PGL [2, 103]. *NF1* is the most common somatic mutation in sporadic PCC, with inactivating *NF1* mutations recently detected in 20–40 % of

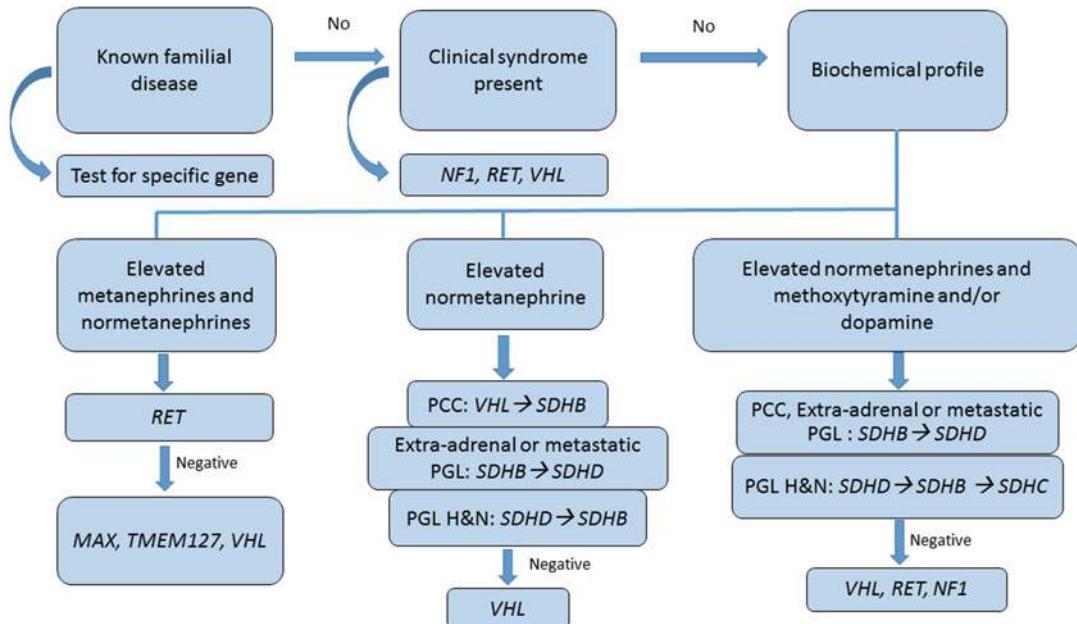


Fig. 5.6 Genetic testing algorithm for patients with pheochromocytomas (PCC) and paragangliomas (PGL). *H&N* head and neck

sporadic PCC [104, 105]. These somatic variants were predominantly truncating and were often accompanied by the loss of the wild-type allele in the tumor [105]. Somatic *NF1* mutations can be found in tumors with somatic *RET* or somatic *VHL* mutations. Somatic *RET* or somatic *VHL* mutations were identified in 14 % of sporadic PCC/PGL [106]. Somatic *HRAS* mutations occur in 7 % of sporadic PCC/PGL [103, 107]. *HRAS* mutant tumors were exclusively found in men; all had benign tumors with increased norepinephrine and/or epinephrine secretion [107]. No somatic *HRAS*, *BRAF* V600E or *TP53* mutations have been found in hereditary PCC/PGL, suggesting that these mutations might be mutually exclusive. The rates of *BRAF* V600E and *TP53* mutations in sporadic PCC/PGL were 1.2 and 2.3 %, respectively [103]. Somatic *HIF2A* mutations were found in 12 % of sporadic PCC/PGL. Tumors with mutated *HIF2A* displayed a pseudo-hypoxic gene expression pattern, similar to those in cluster 1 [108, 109]. Genetic mosaicism of *HIF2A* has been reported in patients with multiple PCC/PGL and congenital erythrocytosis [109].

Genetic Testing

Genetic testing has evolved in the last several years. First-generation sequencing, also known as Sanger sequencing, was introduced in 1977 by Frederick Sanger et al. [110]). This method requires single-stranded DNA template and a DNA primer, along with normal (dNTPs) and modified (either radiolabeled or fluorescently labeled) deoxynucleotides (ddNTPs). The modified ddNTPs incorporated during PCR terminate DNA elongation and resulting DNA fragments can be resolved to provide a DNA sequence. In the late 1990s, next-generation sequencing was introduced and has been used readily over the past 10 years. Similar to Sanger sequencing, DNA is sequenced by synthesis through addition of nucleotides. However, next-generation sequencing platforms are able to process and sequence DNA more quickly at a lower cost. These improvements have made genetic testing more readily available and accessible.

To reduce cost and remain effective, the algorithm for determining germline genetic testing in patients with PCC/PGL should be based

on family history, clinical features of hereditary PCC/PGL, and biochemical profiles. The algorithm is summarized in Fig. 5.6.

- If both metanephhrines and normetanephhrines are elevated:
 - Patients that exhibit no clinical features of neurofibromatosis type I should undergo genetic testing for *RET* proto-oncogene first, followed by *MAX*, *TMEM127*, and *VHL*.
- If methoxytyramine and/or dopamine is elevated:
 - Patients should be tested for *SDHx* mutations (*SDHD* for head and neck PGL first, followed by *SDHB*, and, *SDHB* for PCC, extra-adrenal PGL, or metastatic PCC/PGL first, followed by *SDHD*).
 - Patients with PCC, elevated methoxytyramine and that have no *SDHx* mutations should be tested for *RET*, *VHL*, and *NFI* mutations.
- If normetanephhrines are elevated:
 - Patients with PCC should be tested for *VHL* mutations first, followed by *SDHB*.
 - Patients with PGL should be tested for *SDHx* mutations first (*SDHD* for head and neck PGL first, followed by *SDHB*, and, *SDHB* for extra-adrenal PGL, or metastatic PCC/PGL first, followed by *SDHD*), followed by *VHL*.

Although the earlier algorithm may be cost effective, it is time consuming. A next-generation sequencing strategy has been developed [111] for analysis of germline mutations in individuals with PCC/PGL; this method has a 98.7% sensitivity and rapid turnaround time as it can simultaneously analyze multiple genes [112].

Although the Endocrine Society Clinical Practice guideline for PCC/PGL recommends that all patients with PCC/PGL should be counseled for germline mutation testing [113], the likelihood of *not* having a germline mutation is increased when an individual presents at an older age, without family history, with a unilateral PCC, and with no evidence of metastatic disease. As such, current guidelines caution against rou-

tine testing of germline mutations in these individuals in light of financial costs and limited incremental value of genetic testing in this setting [113]. However, studies are emerging that despite lack of family history, individuals being classified as having sporadic disease may in fact harbor a germline mutation. Neumann et al. showed that in individuals with no family history, a germline mutation could be detected in upward of 24% [3]. In a group of individuals with apparent sporadic disease, as defined by a lack of family history or syndrome and presenting with a single PCC or PGL, germline mutations were found in 14% of individuals, and at a higher rate in those with a PGL compared to single PCC (28.7% vs. 4.5%, respectively). In the same cohort, somatic mutations were found in 43% of individuals [114]. As the cost of genetic testing continues to decrease with the advances in next-generation sequencing methods, routine genetic testing for known germline mutations will likely become more accessible and used in clinical practice.

Management of Patients with PCC/PGL Based on Genetic Information

Management of individuals with PCC/PGL involves a multidisciplinary approach. Once a diagnosis of PCC/PGL is confirmed, germline mutation testing should be obtained prior to a medical or a surgical intervention because the optimal time of intervention, treatment options, and surgical approach may differ based on the risk of malignancy, multifocality, and persistent or recurrent disease. For example, surgical resection of a carotid body tumor in patients with germline *SDHB* mutations should be considered earlier than in patients with *SDHD* mutations because of the higher rate of malignancy and worse disease-free survival [115]. An open approach may be preferable in patients with *SDHB* mutations who present with extra-adrenal PGL to remove surrounding lymph node compartments. A cortical preserving minimally invasive adrenalectomy in patients with *VHL* or MEN2 syndrome should be considered due to the low risk of malignancy; this reduces the risk of

long-term adrenal steroid replacement [116]. The clinical management of other manifestations associated with hereditary PCC/PGL, such as medullary thyroid cancer in patients with MEN2 syndromes, can benefit from genetic information. It is best to obtain genetic testing especially in patients with no obvious family history or apparent clinical syndrome to appropriately counsel the patients regarding the risks of malignancy and recurrent disease as well as the need for post-operative surveillance for PCC/PGL and other manifestations.

A recent guideline from European Society of Endocrinology recommends annual biochemical, anatomical, and functional imaging with plasma or urine metanephhrines to surveil for recurrent disease for at least 10 years. Anatomical imaging may be started 3 months postoperatively and should be continued for a minimum of 10 years. The recommendations are based on data from the European Network for the Study of Adrenal Tumors that demonstrated a 10% risk of recurrence over the first 5 years of follow-up. For patients with a high risk for persistent or recurrent PCC/PGL, such as young patients, those with germline mutations, or patients with malignant (defined as metastasis in lymph nodes or other distant sites) or large PCC/PGL, lifelong surveillance is recommended [117].

For sporadic disease, one may argue that apparently sporadic appearing presentations in individuals in the fourth or fifth decade may need minimal follow-up. However, these individuals may still have 7% risk of recurrent disease over 5 years, compared to 17% in patients with germline mutations [117]. The assessment of younger individuals, from the pediatric population to those in their 30s, remains unclear as they may harbor germline or somatic mutations not yet identified.

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Imaging Modalities for Adrenocortical Tumors

Iuliana D. Bobanga and Christopher R. McHenry

Introduction

Incidental adrenal lesions are being detected with greater frequency due to the widespread use of high-resolution abdominal and thoracic imaging and an increasingly aging population that has a higher incidence of nonfunctioning adrenal adenomas. Tumors of the adrenal gland are detected radiographically in patients who undergo imaging for assessment of biochemical abnormalities, staging of a known primary malignancy, and evaluation of unrelated medical problems [1]. An incidentally discovered adrenal mass >1 cm in size is referred to as adrenal incidentaloma (AI) and is identified in approximately 4–6% of the imaged population [2, 3]. In a large review of autopsy studies, which included over 87,000 autopsies, the overall frequency of an adrenal adenoma was 6%, with a range of 1–32% [4].

The incidence of AIs discovered on computed tomography (CT) increases with age; a rate of 0.2% is documented for patients between 20 and 29 years of age, compared to 7% in patients over 70 years of age [5].

Adrenal lesions can be categorized as benign or malignant, primary or metastatic, and functioning or nonfunctioning. Approximately 95% of AIs originate from the adrenal cortex, the majority of which are benign or nonneoplastic [2]. Ninety-four percent of benign adrenal adenomas are nonfunctioning and approximately 6% autonomously secrete aldosterone, catecholamines, cortisol, or sex hormones [2, 3, 5]. Other miscellaneous adrenal masses include myelolipomas, cysts, hemorrhage, hemangiomas, lymphangiomas, ganglioneuromas, and granulomatous disease. Malignant adrenal lesions make up 2–5% of AIs and have a higher prevalence with increased age [2, 5]. Primary adrenocortical carcinoma is less commonly encountered than metastatic disease. In patients with a known malignancy, an AI represents metastatic disease in approximately 50% of cases [2]. Other rare malignant adrenal lesions include primary lymphoma, hemangiosarcoma, and neuroblastoma [6].

Almost all adrenal masses can be definitively characterized using imaging and biochemical studies alone. Densitometry using unenhanced computed tomography (UCT) and CT contrast medium washout testing, magnetic resonance imaging (MRI) with chemical shift imaging

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(CSI), and fluorine-18 fluorodeoxyglucose positron emission computed tomography ([FDG] PET/CT) are the principal modalities that are used to help differentiate a benign from a malignant adrenal mass. In most patients, a single imaging study is all that is necessary to characterize an adrenal mass and secondary imaging studies are often unnecessary [7]. Selective adrenal vein sampling (AVS) and image guided needle biopsy are other modalities that are rarely used and only for very specific indications. A high incidence of unnecessary imaging that does not affect clinical decision making has been documented in clinical practice [7].

Key elements that help guide management include a prior history of malignancy, the functional status of the mass, and whether the imaging phenotype is suggestive of benign or malignant disease [8]. Adrenalectomy is indicated for a functional adrenal mass, an isolated adrenal metastasis, an adrenal mass ≥ 4 cm, or a mass that has increased in size >0.5 cm in 6 months [7]. Imaging of adrenal masses helps to differentiate benign from malignant lesions [9]. Some adrenal masses have very distinct imaging features that make them easy to characterize with a single imaging modality, while others are more challenging.

CT, MRI, and [FDG] PET/CT are used alone or in combination, depending on the clinical scenario, to characterize an adrenal mass and determine management [7]. Certain adrenal lesions, such as myelolipomas, cysts, and hemorrhage can be definitively diagnosed based on imaging alone and no further evaluation or intervention is required. Table 6.1 presents the imaging phenotype of the most commonly encountered adrenal masses. In this chapter, we will review the imaging modalities used to evaluate adrenal cortical tumors and the specific radiographic characteristics that help distinguish adrenal cortical masses.

Normal Anatomy and Imaging Characteristics

The normal adrenal glands are inverted Y-shaped structures located anterosuperior to the kidney, in the suprarenal space, enclosed by Gerota's fascia.

The bodies of the normal glands measure 10–12 mm in length and the limbs measure 5–6 mm in thickness. The margins are smooth and straight or slightly concave [1]. Normal adrenal glands appear symmetric with homogeneous soft tissue density on CT scan (Fig. 6.1). The right adrenal vein is short and empties into the postero-lateral aspect of the inferior vena cava. Rarely, the right adrenal gland may also be drained by an accessory vein, which empties into the right renal vein or the right hepatic vein. The left adrenal vein empties into the left renal vein. It often joins with the inferior phrenic vein medially to form a common channel, which empties into the left renal vein [1, 10]. The arterial supply to the adrenal glands is more variable and consists of innumerable small arteries arising from the superior, middle, and inferior adrenal arteries, which are branches of the inferior phrenic artery, aorta, and renal artery, respectively. On T1-weighted (T1w) MRI, normal glands are of intermediate signal intensity, while on T2-weighted (T2w) MRI, they are isointense to slightly hypointense compared to the liver. On [FDG] PET, the normal uptake in the adrenal glands varies from no uptake to moderate uptake, and is usually similar to or less than background liver [1].

Principles of Adrenal Imaging

Most adrenal lesions can be accurately characterized into benign and malignant categories based on the results of modern imaging techniques alone. Image-guided needle biopsy is rarely necessary and is usually limited to patients with a known primary malignancy and a solitary adrenal mass less than 4 cm to distinguish a nonfunctioning adenoma from a metastasis. The key characteristics that can be delineated from imaging of the adrenal glands include lesion size, morphology, lipid content, intravenous contrast washout characteristics, and metabolic activity [11].

The size of an adrenal lesion is directly related to the risk of malignancy [2, 5]. In a report of 887 patients with AIs, a diameter larger than 4 cm had a 90% sensitivity for predicting adrenocortical

Table 6.1 Imaging phenotype of various adrenal lesions (adapted from [2, 5, 39])

Adrenal lesion (frequency of AI)	Size	Shape and texture	Growth rate	High vascularity?	Unenhanced CT HU	15 min CT washout	MRI	PET
Adenoma (50–80 %)	Small <3 cm	Round, oval, smooth margins, homogeneous	Slow, <1 cm per year	No	≤10 HU in 70 %	RPW >40, APW >60	CSI—Loss of signal intensity in out-of-phase imaging. T2w—isointense with liver	Negative
ACC (<5 %)	Large, >4 cm	Irregular, heterogeneous, mixed densities due to necrosis and calcifications	Rapid, >2 cm per year	Yes	>10, usually >25 HU	RPW <40	T2w—hyperintense compared to liver. Heterogeneous due to hemorrhage and necrosis	Positive
Pheochromocytoma (5 %)	Large, >3 cm	Round, oval, smooth margins, heterogeneous with cystic areas and hemorrhage	Slow, 0.5–1 cm per year	Yes	>10, usually >25 HU	RPW <40	T2w—markedly hyperintense compared to liver	Usually positive
Metastasis (2.5 %)	Varies, <3 cm	Oval or irregular, unclear margins, heterogeneous, mixed densities, occasional hemorrhage	variable	Yes	>10, usually >25 HU	RPW <40	T2w—hyperintense compared to liver	Positive
Myelolipoma (5–10 %)	1–5 cm	Smooth, round, variable with macroscopic fat	slow	No	<0, often <-50		High signal intensity on T1w	Negative
Hematoma (1 %)	Variable	Smooth	Rapid		>10 HU, sometimes >50 HU		Variable	Negative
Cyst (1 %)	Variable	Smooth, round	stable			Does not enhance	T2w—hyperintense compared to liver	negative



Fig. 6.1 CT image of a normal left adrenal gland in the axial plane (arrow)

carcinoma, but poor specificity; only 24 % of lesions over 4 cm were malignant [6]. The risk of malignancy is 2 % for an adrenocortical lesion ≤ 4 cm, 6 % for a lesion 4.1–6 cm, and 25 % for lesions >6 cm. [12].

Size is also predictive of tumor stage and prognosis in adrenocortical carcinoma [5, 13]. Change in size over time is a significant feature that aids in diagnosis and management of an adrenal lesion. Stability of a lesion over a 6-month period of time or longer suggests a benign process, whereas increase in size of >0.5 cm warrants additional investigation and treatment [5]. However, not all enlarging adrenal masses represent malignancy. Benign adrenal adenomas can grow slowly over time. Hemorrhage into an adrenal gland, either traumatic or spontaneous (as in myelolipoma) can cause abrupt adrenal enlargement [2].

Morphologic features that increase concern for a malignant adrenal neoplasm include irregular margins, hemorrhage, and necrosis. Lesions with smooth borders are more likely to be benign. Simple adrenal cysts usually can be characterized morphologically based on their uniform and homogenous nature while complex ones are more difficult to distinguish from other diagnoses [2]. The lipid content and intravenous washout characteristics of adrenal lesions can be evaluated with CT or MRI, while metabolic activity is evaluated with PET imaging.

Computed Tomography (CT)

CT is used to characterize the size, morphology, lipid content, and perfusion of adrenal lesions. CT for evaluation of an adrenal mass is a multi-phase study, which includes an unenhanced scan, a 1-min dynamic contrast-enhanced scan, and a 15-min delayed-enhanced scan, reconstructed in 2–3 mm cuts in axial and coronal planes [10]. The majority of adrenal adenomas can be characterized based on the features seen on the unenhanced CT (UCT) using CT densitometry. Because there is an inverse relationship between the fat content and attenuation on UCT, lipid-rich adenomas have a lower attenuation value (average -2.2 HU) compared to nonadenomas (28.9 HU) [14, 15]. To measure densitometry, a region of interest (ROI) is placed over the adrenal gland, including the center of the lesion over one-half to two-thirds of the surface area, avoiding necrotic or hemorrhagic areas, and the Hounsfield units are measured [2, 15]. The location of the ROI, particularly for adrenal lesions >3 cm, significantly affects the sensitivity and specificity of CT densitometry for differentiating a large adenoma from a carcinoma. To minimize loss of CT sensitivity for large adenomas and carcinomas, a ROI that covers more than half of the mass has the highest sensitivity and specificity [16]. A threshold of 10 HU for characterization of an adrenal adenoma has a sensitivity of 71 % and specificity of 98 % [17]. Because of the high specificity, an adrenal mass <10 HU on UCT does not require additional evaluation with intravenous contrast. This is important to avoid additional radiation and reduce the cost.

Up to 30 % of adrenal lesions are lipid poor with unenhanced CT attenuation values that are >10 HU. These adrenal lesions are classified as indeterminate and require further characterization with contrast enhanced CT (CECT) to evaluate perfusion and washout of contrast from the lesion. Contrast washout is more rapid for benign than malignant lesions [11]. By measuring the ratio of adrenal attenuation on the washout-delayed scan at 10 or 15 min when compared with the initial dynamic enhanced study at 1 min,

adrenal lesions can be characterized with great precision [18, 19]. If an UCT was obtained, absolute percentage washout (APW) can be calculated (Table 6.2). If an UCT is not available (as in a patient who underwent a CECT for another indication), the relative percentage washout (RPW) can be calculated (see Table 6.2). Both measurements can accurately differentiate an adenoma from a malignancy [20, 21]. UCT histograms and dual energy UCT are other applications that are being used for evaluation of an adrenal mass. CT histograms have been shown to increase the sensitivity for characterizing an adenoma [22]. Neither application, however, is in common use.

The advantages of CT for evaluating an adrenal mass are that it is readily available, rapidly performed, and effective for characterizing a mass based on lipid content and contrast washout. The drawbacks of CT include contrast allergy, renal insufficiency, and radiation exposure, which confer a small but increased risk of cancer and is of particular concern for young patients. In patients with adrenal masses >10 HU, further characterization can be obtained by CECT or by MRI with chemical shift imaging (CSI). When comparing the two modalities, 15-min delayed CECT was more accurate than MRI with CSI in differentiating adrenal masses with an attenuation value >10 HU as benign or malignant [23]. The sensitivity of MRI with CSI decreases as the CT attenuation of adenomas increases. Furthermore, the likelihood of an etiology other than a nonfunctioning adenoma, including malignancy, increases almost threefold for every 1 cm increase in size and 10 HU increase in attenuation [23].

Table 6.2 Washout computed tomography calculations of the absolute percentage washout (APW) and relative percentage washout (RPW)

$APW = \frac{[ECT(HU) - DCT(HU)] \times 100}{[ECT(HU) - UCT(HU)]}$
$RPW = \frac{[ECT(HU) - DCT(HU)] \times 100}{ECT(HU)}$

ECT early contrast-enhanced CT, *DCT* delayed enhanced CT, *UCT* unenhanced CT, *HU* Hounsfield units

Magnetic Resonance Imaging (MRI)

MRI is effective in characterizing soft tissue masses without the use of ionizing radiation. It is more costly than CT but may be preferable in children and adolescents, women of childbearing age, and patients with contrast allergies or renal insufficiency. The main application of MRI that is used for evaluation of an adrenal mass is CSI, an effective method to identify fat [11]. CSI differentiates fat and water molecules in tissue. As patients are placed in a strong magnetic field, protons rearrange their axes and spin. When a radiofrequency wave is applied at the same frequency as the spinning protons, this energy is absorbed. When the radiofrequency wave is turned off, the protons give off energy as they return to their original state, which is captured as images and varies based on the chemical structure of the tissue. Water and fat protons have a different resonance frequency required for a change in the orientation of their rotational axis. Because they are spinning at different frequencies, water and fat protons are only in sync, or “in phase” every few milliseconds, and then are completely opposite, or out of phase in half that time. When they are out of phase, fat and water protons cancel each other out within a voxel on the MR images obtained, which results in loss of signal intensity on out-of-phase imaging (lipid-rich adenomas appear dark on out-of-phase imaging) compared to in-phase imaging. Thus, the amount of signal intensity decrease will depend on the fat-to-water proton ratio within a voxel [2, 11, 24]. This characteristic makes MRI with CSI a useful imaging modality for differentiating lipid-rich adenomas from other lesions.

MRI with CSI has a similar sensitivity and specificity to UCT, but may have an advantage in evaluating lesions less than 30 HU. Above this threshold, the sensitivity and specificity of CSI decreases and lipid-poor adenomas may be indeterminate [25, 26]. The chemical shift occurrence can be quantified by measuring the signal intensity index (SII) and the adrenal-to-spleen ratio (ASR) (Table 6.3). However, because they are cumbersome to calculate, these quantitative techniques are not routinely used in clinical practice.

Radiologists rely more on the visual characteristics of the chemical shift, which is shown to be as effective as quantitative methods [2, 27].

Gadolinium-enhanced MRI can also be obtained for further characterization. Similar to CECT, benign adrenal adenomas have prompt, but weak enhancement and rapid washout, while malignant lesions have marked enhancement and slower washout [11]. Despite these general characteristics, gadolinium-enhanced imaging has not been shown to be reliable for characterization of adrenal masses [28]. With conventional spin-echo MRI, T1-weighted (T1w) and T2-weighed (T2w) images are obtained. An adenoma can be differentiated from a pheochromocytoma, which typically has a high signal intensity T2w image, referred to as a “light bulb” sign [11]. However, there is significant overlap in the characteristics of adenomas and nonadenomas using conventional spin-echo MRI with T1w and T2w imaging and, as a result, it is not routinely used for characterization of adrenal cortical tumors. Conventional MRI plays a key role in determining the origin of a large retroperitoneal mass and staging of malignant adrenal tumors [28]. Other MRI techniques, including diffusion-weighted imaging, dynamic contrast-enhanced imaging, and spectroscopy are not routinely used in the evaluation of adrenal lesions because they have a poor sensitivity for characterizing adenomas [22].

Table 6.3 Chemical shift imaging magnetic resonance imaging parameters

$\text{SII} = \frac{\text{SI}_{\text{IP}} \text{adrenal mass} - \text{SI}_{\text{OP}} \text{adrenal mass} \times 100}{\text{SI}_{\text{IP}} \text{adrenal mass}}$
$\text{ASR} = \frac{\frac{\text{SI}_{\text{OP}} \text{adrenal mass}}{\text{SI}_{\text{OP}} \text{spleen}}}{\frac{\text{SI}_{\text{IP}} \text{adrenal mass}}{\text{SI}_{\text{IP}} \text{spleen}}}$

SII signal intensity index, ASR adrenal-to-spleen ratio, SI_{IP} signal intensity, in phase, SI_{OP} signal intensity, opposed phase

Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography ([FDG] PET)

A PET scan is used to evaluate the metabolic activity of tissue. Most malignant lesions will have increased uptake of radiolabeled glucose analog related to the increased glycolysis that occurs in cancer cells compared to normal cells [29]. The role of PET imaging for adrenal cortical tumors is typically limited to patients with a known extra-adrenal malignancy or adrenocortical carcinoma to determine if there is metastatic spread. It is also of value in oncologic patients who have an AI that is suspicious for malignancy. In order to decide on the appropriate management of an adrenal metastasis, it is important to establish whether or not the adrenal gland is the only site of metastatic disease. A solitary adrenal metastasis can be treated with adrenalectomy, whereas disseminated metastatic disease is treated nonsurgically.

The accuracy of [FDG] PET to distinguish benign and malignant adrenal disease is excellent (98 %), with a specificity of 97 % and a sensitivity of 91 % based on a meta-analysis of 21 studies [30]. FDG uptake that is less than the liver is characteristic of a benign lesion and FDG uptake that is the same or greater than the liver is characteristic of a malignant lesion. It has been reported that approximately 5 % of adrenal adenomas will have FDG uptake similar or greater than the liver and are a cause for false-positive PET scans [31]. The specificity of [FDG] PET increases when only lesions that have greater uptake than the liver are considered malignant.

In the majority of cases, an adrenal mass visualized on [FDG] PET does not require subsequent CT or MRI to further characterize the lesion. Multiple studies have shown the accuracy of [FDG] PET in differentiating benign and malignant adrenal disease [30, 32–35]. [FDG] PET can be obtained in combination with CT imaging, giving an additional level of anatomic and morphologic data, allowing for CT densitometry and washout data to be incorporated in the analysis and increasing the diagnostic accuracy of the test to almost 100 % [34].

In clinical practice, [FDG] PET imaging of an adrenal lesion is only required if prior imaging studies such as CT densitometry or washout analysis are inconclusive [2, 7]. Standard uptake values (SUVs) and the standardized uptake ratio (a quantitative value of the adrenal signal compared with the liver) is a semiquantitative parameter that helps quantify [FDG] PET findings [34, 36]. A false-positive rate of 5–16% is reported for adrenal lesions on PET CT due to significant FDG uptake in some adenomas, endothelial cysts, and inflammatory and infectious lesions [5, 34]. Those lesions often display mild FDG avidity compared to the high FDG avidity of malignant lesions, and should be considered indeterminate and additional imaging should be performed to further characterize them [36]. Malignant lesions can also be missed on [FDG] PET due to small size <1 cm, those with significant hemorrhage or necrosis, or metastatic lesions from primary tumors that are not PET avid (carcinoid, pulmonary bronchoaleveolar carcinoma) [34].

¹¹C-metomidate (MTO)—PET can be used to differentiate a primary malignant adrenal lesion from metastatic disease [37]. The radiotracer ¹¹C-metomidate is a potent inhibitor of 11 β -hydroxylase and aldosterone synthase, and is a specific marker of adrenocortical neoplasms. MTO-PET has recently been evaluated as an alternative test to adrenal venous sampling in lateralizing aldosterone-secreting adenoma [38]. Both the lack of data to support routine use as well as lack of widespread availability and high cost limits the common use of MTO-PET [5].

Adrenal Cortical Tumors and Imaging Features

Cortical Adenoma

The most common incidentally discovered adrenal lesion is a benign adrenal cortical adenoma, most of which are nonfunctioning [2, 9]. Functioning and nonfunctioning adrenal cortical adenomas have a similar radiographic appearance and are differentiated by biochemical evaluation. An adrenal adenoma is typically a well-circum-

scribed, homogeneous solid, encapsulated mass of variable size with smooth regular borders and a high lipid content [39]. On histopathology, an adrenal cortical adenoma consists of cells with pale-staining lipid-rich cytoplasm. Approximately 70% of adrenal cortical adenomas have a lipid-rich cytoplasm, whereas 30% are lipid poor [15, 40]. On UCT, the density measurements are inversely proportional to the lipid content of a lesion. Lipid-rich adenomas are usually homogeneous lesions with regular margins and a density less than 10 HU on UCT (Fig. 6.2) [1, 10].

A meta-analysis found that lipid-rich adenomas can be accurately diagnosed with a noncontrasted CT scan, using an attenuation cutoff of 10 HU or less, with a 71% sensitivity and 98% specificity [17]. Thus, lipid-rich adenomas can be definitively diagnosed on a noncontrasted CT scan if they are both homogeneous and less than 10 HU. However, up to 30% of adenomas are lipid poor and may be hard to differentiate from a primary malignancy or a metastasis on both UCT and MRI with CSI [5, 41]. An attenuation value greater than 10 HU for an adrenal mass is considered indeterminate and additional imaging evaluation, with CT contrast washout testing, MRI with CSI or PET-CT should be performed depending on the clinical scenario [1].

CT contrast washout studies help further differentiate adrenal lesions. Washout values can be calculated using a triple phase adrenal CT scan with intravenous contrast, which has an unenhanced



Fig. 6.2 Unenhanced CT image of a lipid-rich left adrenal adenoma in the axial plane (arrow)

phase, a 1-min enhanced phase, and a 10 or 15-min delayed enhanced phase. Using the formulas given in Table 6.2, the absolute percentage washout (APW) and relative percentage washout (RPW) can be calculated [10, 22]. Almost all adenomas are characterized by rapid enhancement reaching a peak within 1 min and enhancing up to 80–90 HU with rapid wash out of more than 50% of the contrast on delayed scans, whereas primary adrenocortical carcinoma, pheochromocytoma, and metastases retain contrast and do not wash out. Pheochromocytomas in particular enhance to more than 100 HU, which distinguishes them from adenoma [42]. An APW greater than 60% or RPW greater than 40% have a sensitivity of 88–96% and a specificity of 96–100% for diagnosis of an adrenal adenoma; however, this can vary based on the size of the lesion [20]. Adenomas >3 cm in size are often more heterogeneous, and thus CT sensitivity for a benign adenoma decreases, making it difficult to differentiate a large adenoma from a carcinoma [16, 22].

CSI is a fast and reliable MRI sequence for characterizing lipid-rich adenomas [22, 43]. As a result of intracellular lipid and water protons within the same imaging voxel and the frequency difference between lipid and water, lipid-rich adenomas appear hyperintense on in-phase imaging and hypointense on out-of-phase imaging [22]. It is the loss of signal intensity on out-of-phase imaging that characterizes lipid-rich adenomas. The advantage of MRI with CSI compared to noncontrast CT scan in identifying adrenal adenomas is that it has a higher sensitivity for intracellular lipid and there is no exposure to ionizing radiation [43]. The adrenal-to-spleen ratio (ASR) and the signal intensity index (SII) are useful quantitative MRI parameters (see Table 6.3). Values of ASR <0.71 and SII >16.5% are diagnostic of an adrenal adenoma [44].

The sensitivity of MRI with CSI varies based on UCT findings. For an adrenal lesion <20 HU on UCT, the sensitivity for adenoma on CSI is as high as 100%, compared to 64% for adrenal lesions >20 HU [44]. In patients with an indeterminate adrenal mass that has an attenuation value of 10–20 HU on an UCT, MRI is preferable to

CT contrast washout testing in patients with renal insufficiency, a contrast allergy, or in young patients to avoid radiation exposure. CT contrast washout testing, because of its higher sensitivity in characterizing lipid-poor adenomas, is preferable for evaluation of adrenal lesions with an attenuation value >20 HU on UCT [44].

[FDG] PET can help differentiate benign from malignant adrenal disease. It is important in patients with a known malignancy and a suspected or known adrenal metastasis to exclude other sites of metastatic disease. The most commonly used radiopharmaceutical is fluorine 18-fluorodeoxyglucose ¹⁸F [FDG]. In quantitative analysis of FDG uptake, there is some overlap between adenomas and malignant lesions, but generally the uptake is less than the liver for an adenoma and other benign lesions, and equal to or greater than liver for a malignant lesion [22]. There are rare benign adenomas that have FDG uptake equal to or greater than the liver and they account for the 5% false-positive lesions reported on [FDG] PET imaging [22]. In a meta-analysis, ¹⁸F [FDG] PET or PET/CT had a sensitivity of 97% and a specificity of 91% in distinguishing benign from malignant adrenal lesions [30].

Recommendations for follow-up of patients with a benign nonfunctioning cortical adenoma are primarily based on expert opinion and are likely to evolve over time. The American Association of Clinical Endocrinologists and the American Association of Endocrine Surgeons guidelines for management of AIs suggest that patients with a nonfunctioning adrenal mass <4 cm with an imaging phenotype consistent with an adenoma need to have radiographic reevaluation at 3–6 months and then annually for 1–2 years and repeat hormonal evaluation annually for 5 years [8]. Subclinical Cushing's syndrome is the most common functional disorder that can develop in a previously hormonally inactive adenoma. Adrenalectomy should be considered for an adrenal mass that increases in size by 0.5 cm in 6 months or 1 cm in a year or an adrenal mass that becomes hormonally active [7, 8].

Aldosterone Producing Adenoma

Approximately 1% of incidental adrenal lesions are aldosterone-producing adenomas (APA) [5]. APA is the cause for approximately 30–35% of primary hyperaldosteronism; bilateral adrenal hyperplasia accounts for 60–65% of cases [45]. Unilateral hyperplasia, angiotensin II responsive adenomas, aldosterone-producing adrenal cortical carcinoma, familial hyperaldosteronism type I (glucocorticoid-remediable hyperaldosteronism), and types II and III are infrequent causes of primary hyperaldosteronism [45]. Patients with primary hyperaldosteronism have hypertension that is often severe, requiring 3 or more antihypertensive agents and they may also have associated hypokalemia [8, 45, 46]. Unilateral forms of primary hyperaldosteronism can be cured by adrenalectomy.

A combination of laboratory and imaging studies is required to establish whether or not primary hyperaldosteronism is surgically correctable. This usually consists of measurement of a serum aldosterone to plasma renin activity ratio, confirmatory testing with a saline load test, and a high resolution CT scan of the adrenal glands. The gold standard for distinguishing a unilateral from bilateral hormone overproduction is adrenal venous sampling (AVS) [46]. APAs have a mean diameter of 1.5–2 cm and are usually solitary, but on rare occasions can be bilateral [47]. Primary unilateral adrenal hyperplasia usually consists of micronodular or macronodular hyperplasia, which suggests a continuum between adrenal adenoma and hyperplasia and explains the diagnostic challenge and need for AVS for subtype evaluation [8].

In primary hyperaldosteronism, aldosterone oversecretion is largely independent of the renin-angiotensin system. The screening test of choice in patients with suspicion for primary hyperaldosteronism is the aldosterone/renin ratio (ARR), calculated using the plasma aldosterone concentration (ng/dL) to plasma renin activity (ng/mL) ratio (PAC/PRA) [8, 45, 46, 48]. Ratios are laboratory dependent and vary at each institution, but in general a ratio >20:1 is considered abnormal [45]. Several confounding factors need to be

dealt with to ensure the validity of the results including: discontinuation of mineralocorticoid receptor blockers 4–6 weeks prior to the test and performing the test with certain standards regarding posture, time of day, and potassium repletion status [46]. If the ARR is suggestive of primary hyperaldosteronism, a confirmatory test is required to establish the diagnosis. The three most commonly used tests are the intravenous saline loading test, the oral saline loading test, and the fludrocortisone-suppression test [46].

To differentiate the subtypes of primary hyperaldosteronism, CT scanning is the imaging modality of choice recommended by the current available guidelines [8, 46, 49]. High resolution CT with 3 mm slices and an adrenal protocol consisting of unenhanced imaging followed by administration of intravenous contrast is obtained. APAs are usually small, varying in size from 0.5 to 2.0 cm, and have a mean attenuation value of –2.2 HU [14]. However, patients with surgically correctable primary hyperaldosteronism may have normal appearing adrenal glands, a nodule in one adrenal gland with a thickened contralateral adrenal gland (Fig. 6.3), multiple nodules in one adrenal gland, or bilateral adrenal nodules.

Several studies have examined the efficacy of CT and MRI in detecting APAs in patients with biochemically confirmed primary hyperaldosteronism and found CT sensitivity and specificity to be 53–100% and 33–100%, respectively, and MRI sensitivity and specificity of 74–100% and 64–92%, respectively [50]. While CT scanning is the most widely used imaging technique in the workup of both incidental adrenal nodules and primary hyperaldosteronism, it does have some limitations. CT is not always accurate in differentiating an APA from bilateral adrenal hyperplasia [51]. Its lack of optimal sensitivity for detecting an APA is due to three main challenges: difficulty in identifying APAs less than 1 cm, lack of specificity when both an APA and a contralateral nonfunctioning adenoma are present (see Fig. 6.3), and incorrectly diagnosing a dominant nodule in bilateral adrenal hyperplasia as an APA [46, 51]. These challenges become more significant with advancing age, as the frequency of AIs increases.

AVS is the best modality for differentiating unilateral from bilateral adrenal hyperaldosteronism. In a study of 203 patients with primary hyperaldosteronism from the Mayo clinic, 95 % underwent AVS in addition to CT imaging. On the basis of CT findings alone, 21 % of patients would

have been incorrectly excluded as candidates for adrenalectomy and 25 % of patients would have had unnecessary or inappropriate adrenalectomy [52]. The authors concluded that AVS is an essential diagnostic step in the majority of patients with primary hyperaldosteronism to distinguish

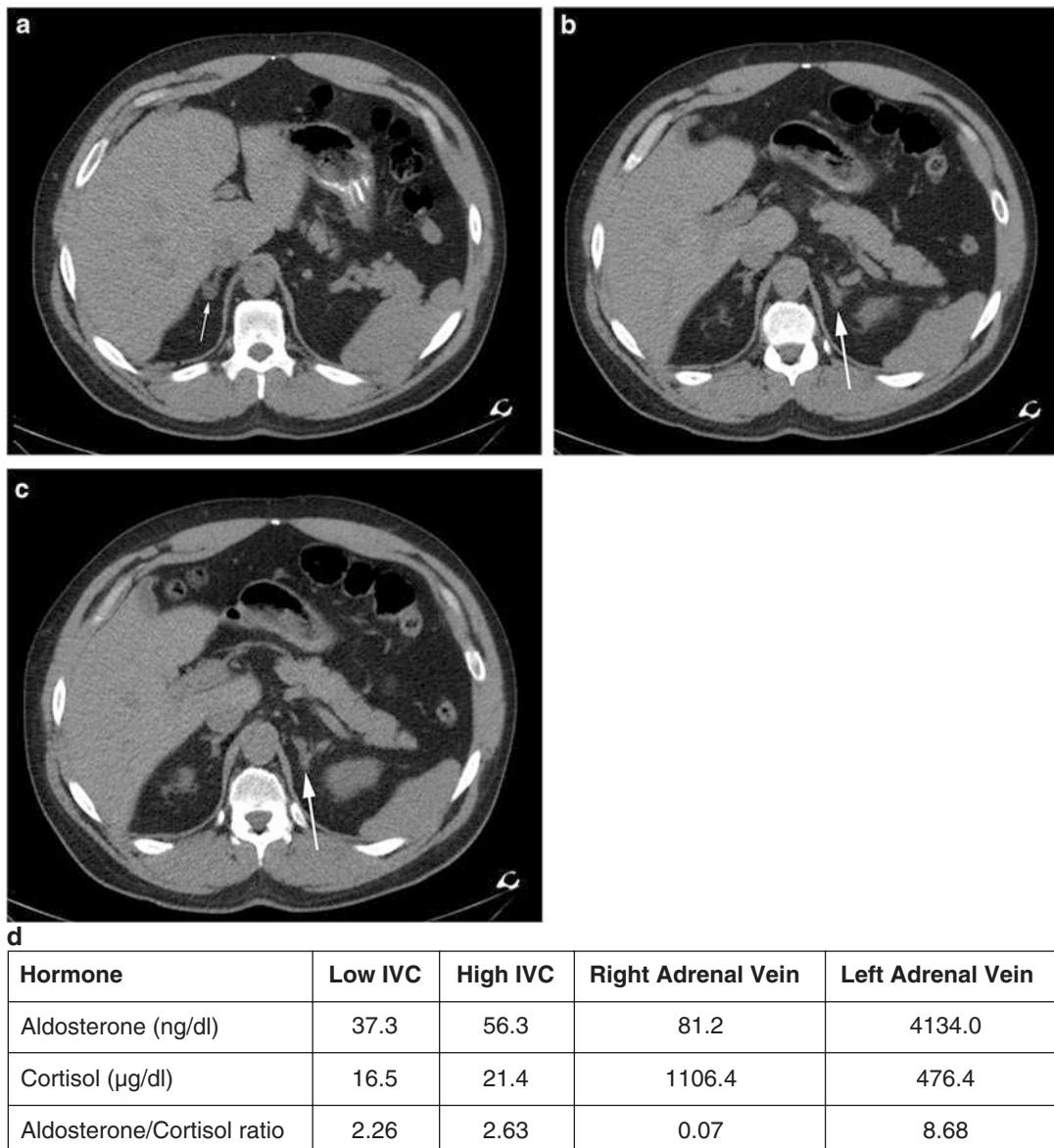


Fig. 6.3 (a) CT image demonstrating a 1.4 cm right adrenal mass, which measured 3 HU (arrow) in a 48-year-old patient with primary hyperaldosteronism. (b, c) CT images from the same patient demonstrating increased thickness of the left adrenal gland without a well-defined

mass. (d) The results of adrenal vein sampling, completed using continuous cosyntropin stimulation, revealed lateralization of aldosterone hypersecretion to the left adrenal gland. The patient's hyperaldosteronism resolved with laparoscopic left adrenalectomy. *IVC* inferior vena cava

between unilateral and bilateral aldosterone hypersecretion (see Fig. 6.3), with the exception of patients less than 40 years of age with CT imaging that reveals a solitary unilateral adenoma larger than 1 cm and a morphologically normal contralateral adrenal gland. These patients had high biochemical and clinical cure rates and AVS is unnecessary [8, 52]. In patients with primary hyperaldosteronism who refuse surgery or who are not appropriate surgical candidates, AVS can be omitted and they should be managed medically with aldosterone antagonists.

AVS is a challenging procedure primarily due to the difficulty in cannulating the right adrenal vein. Successful AVS is highly dependent on operator experience. The success rate for cannulating the right adrenal vein has been reported to be 74% in a systematic literature review of 47 reports [45, 53], but with increased experience at high-volume centers, it is noted to be greater than 95% [52, 54]. Prior to performing AVS, patients must discontinue eplerenone and spironolactone 4 or 6 weeks prior to the procedure, respectively. While rare (<1% of cases), aldosterone-and-cortisol cosecreting adenomas have been described. Because unilateral overproduction of cortisol can greatly affect the AVS results, exclusion of subclinical hypercortisolism should be performed prior to AVS [46]. Based on the individual institution protocol, cosyntropin may be used before or during the procedure to augment the aldosterone-to-cortisol ratio in patients with APA. Once cannulation of the adrenal veins is achieved, the aldosterone and cortisol concentration is measured in each adrenal vein. The aldosterone-to-cortisol ratio on one side is then divided by the aldosterone-to-cortisol ratio on the other side. While specific cutoffs vary by institution, generally a ratio >4:1 is indicative of a unilateral source of aldosterone excess, likely responsive to unilateral adrenalectomy [8, 45].

Cortisol-Producing Adenoma

Cortisol-producing adenomas are the most frequent functional adrenal gland tumors, comprising 5.3% of incidentally found adrenal nodules

[5]. Cortisol oversecretion due to a primary tumor of the adrenal gland results in either overt or sub-clinical Cushing's syndrome (SCS). Incidentally found adenomas are more likely to cause SCS than those found during workup for Cushing's syndrome [8]. Explicit clinical signs and symptoms of Cushing's syndrome are not always present, underscoring the importance of laboratory screening to make a diagnosis of Cushing's syndrome and confirmatory tests to establish the specific cause (central, peripheral, or rarely ectopic).

Cushing's syndrome is caused by primary adrenal hypercortisolism in approximately 20% of patients and is due to an adrenal cortical adenoma, adrenal cortical carcinoma, or primary adrenocortical hyperplasia [55]. Screening and confirmatory tests in the workup for Cushing's syndrome are used to assess for the three pathophysiological derangements: (1) excess production of cortisol (24 h urine free cortisol test); (2) autonomous cortisol secretion despite inhibition of adrenocorticotrophic hormone (ACTH) secretion (dexamethasone suppression testing); and (3) loss of the normal diurnal pattern of cortisol secretion, with abnormally high late night cortisol secretion (late night cortisol secretion test) [8, 40, 55].

Once a biochemical diagnosis of Cushing's syndrome is made, the underlying subtype must be differentiated with radiographic studies to determine optimal treatment. CT is the imaging modality of choice to localize and differentiate a cortical adenoma from adrenocortical carcinoma or primary micronodular or macronodular hyperplasia as the underlying pathologic cause of Cushing's syndrome [55–58].

Radiologic findings of adrenal hyperplasia are nonspecific, showing either enlarged or normal-sized glands. In rare cases of primary pigmented nodular adrenocortical disease (PPNAD), imaging may show multiple small hypodense nodules <5 mm [58, 59]. In ACTH-independent macronodular adrenal hyperplasia (AIMAH), the adrenal glands have massive bilateral enlargement with multiple nonpigmented ginger-like nodules ranging from 1 to 5 cm [58, 60]. Cortisol-producing adenomas have imaging features similar to other benign nonfunctioning adrenal

cortical adenomas; they are homogeneous, smaller than 4 cm, with smooth borders, <10 HU on UCT, and have an APW >60 % on CT washout studies [39, 58]. There may also be contralateral adrenal atrophy related to adrenal cortical thinning from cortisol suppression of ACTH secretion [61].

Myelolipoma

Myelolipomas are rare benign adrenal lesions composed of macroscopic fat and hematopoietic tissue (mostly myeloid and erythroid cells) [39]. They are nonfunctional and typically asymptomatic, with only large myelolipomas manifesting pain or rarely, retroperitoneal hemorrhage [40]. They have a characteristic imaging phenotype identified due to the macroscopic fat within the mass. On UCT, the macroscopic fat of a myelolipoma is similar in attenuation to retroperitoneal fat, and a well-circumscribed lesion is seen with low attenuation <0 to -30 HU (Fig. 6.4). Noncontrasted CT is more sensitive in diagnosing a myelolipoma than contrast-enhanced CT (CECT), due to the possible pseudoenhancement effects that can occur with CECT [1]. On MRI, myelolipomas are hyperintense on T1w and T2w images without fat suppression and hypointense on T1w and T2w images with fat suppression. On T1w opposed-phase images, a characteristic India ink artifact is seen at the fat–water interfaces of the mass that is pathognomonic for myelolipoma [1]. Pseudocapsules are common and calcifications are found in 24 % of adrenal myelolipomas [10]. There is no avid FDG uptake on PET scan [39].

Cysts and Pseudocysts

Adrenal cysts and pseudocysts are rare benign lesions usually found incidentally. They appear as well-defined homogeneous nonenhancing lesions. On CT, they measure 0–20 HU, consistent with water density (Fig. 6.5). On MRI, they have fluid signal intensity. MRI is useful for establishing a definitive diagnosis of cyst (see

Fig. 6.5). Adrenal cysts do not enhance internally with contrast, although their thin wall may enhance [10]. They can occasionally have peripheral calcifications or internal septations. Complicated cysts have areas of irregularity and wall thickening that do not enhance. If thick or nodular septal enhancement is present within a cystic adrenal mass, the lesion requires resection [1]. Adrenal pseudocysts result from previous episode of hemorrhage and can appear as complex cysts with higher density, internal septations, and calcifications [10].

Adrenal Cortical Cancer

Adrenal cortical carcinoma (ACC) (Fig. 6.6) is a rare malignancy, with an incidence of 1–2 cases per million people a year [10, 39]. It is an aggressive cancer for which early diagnosis and treatment greatly influences prognosis. Most patients with ACC present with signs and symptoms of steroid hormone excess or abdominal fullness due to mass effect, but 15 % of patients with ACC are asymptomatic and diagnosed incidentally [62]. ACCs make up 4.7 % of all AIs [5]. While ACCs grow faster than benign lesions, they may not grow rapidly. The goal of an adrenal incidentaloma screening protocol is to identify all



Fig. 6.4 Large right adrenal myelolipoma seen incidentally on contrast-enhanced CT

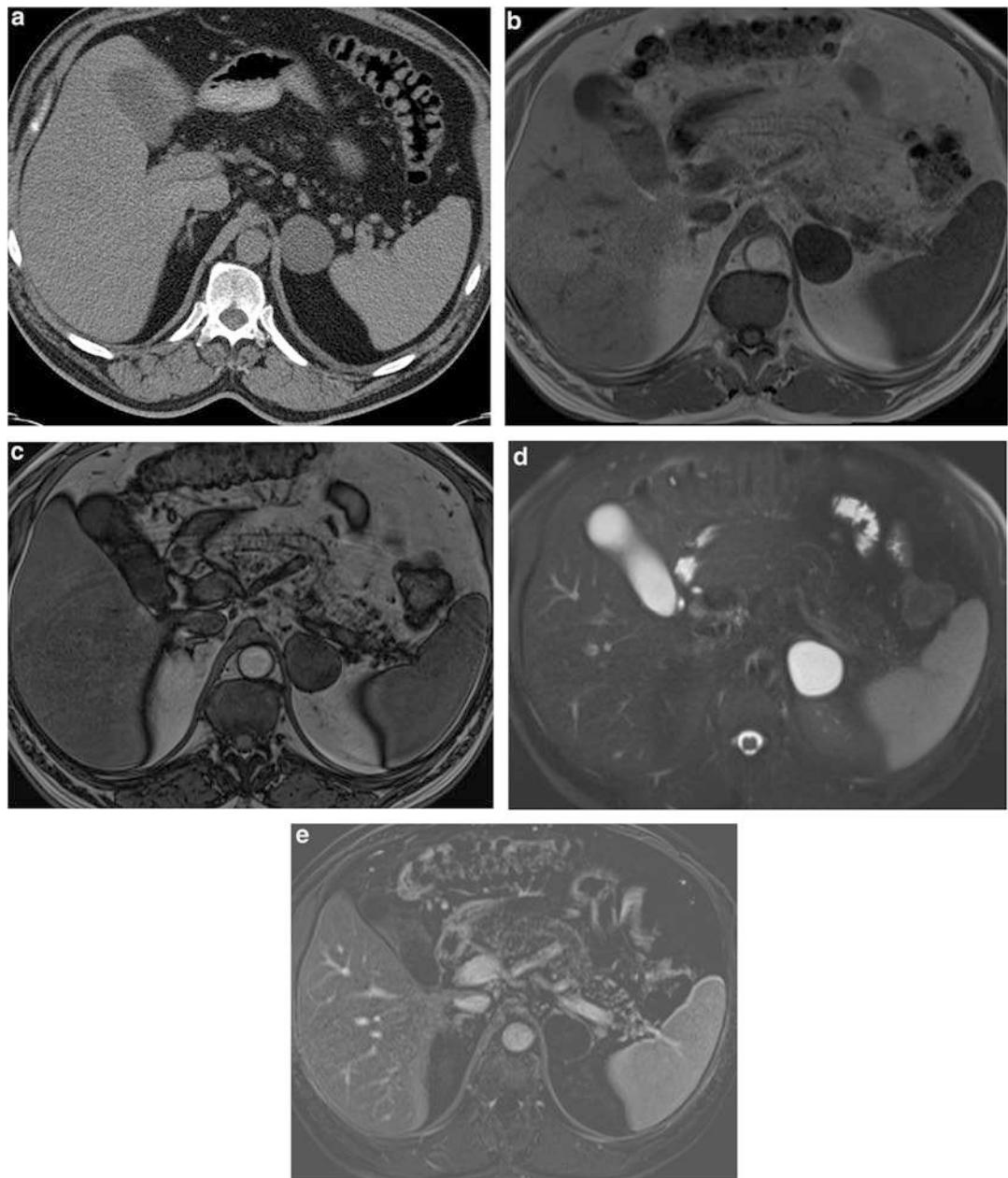


Fig. 6.5 Adrenal cyst appearance on unenhanced CT and various MRI sequences. (a) 4.7 cm homogeneous hypodense left adrenal mass that measures between 16 and 20 Housefield units on unenhanced CT. (b) In-phase T1w MRI without contrast of the left adrenal mass. (c)

Out-of-phase MRI with no loss of signal in the left adrenal mass compared to the in-phase image. (d) Left adrenal mass appears bright on T2w image. (e) MRI postcontrast image of the mass, showing no contrast uptake, confirming the diagnosis of an adrenal cyst

patients with ACC as early as possible. Recommended guidelines suggest radiographic reevaluation of an AI at 3–6 months and then annually for 1–2 years with hormonal evaluation

at the time of diagnosis and annually for a total of 5 years [8].

The prevalence of ACC within all AIs depends on the size of the tumor: 2% of adrenal lesions

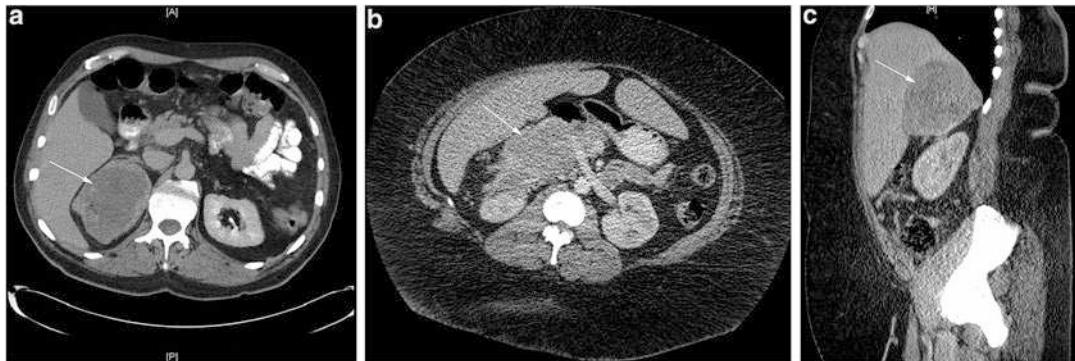


Fig. 6.6 Adrenal cortical carcinoma. (a) CT image of a right adrenal cortical carcinoma in the axial plane with central necrosis (arrow). (b) Right adrenocortical carcinoma (arrow) with irregular margins causing duodenal

displacement and possible invasion of the duodenum and right kidney. (c) Right adrenocortical carcinoma in the coronal plane (arrow) and possible hepatic invasion

≤ 4 cm, 6% of lesions 4.1–6 cm, and 25% of lesions ≥ 6 cm are ACCs [12]. The national Italian study group on adrenal tumors determined the size cutoff of 4 cm was associated with a 93% sensitivity and 24% specificity for malignant disease [6]. Sixty percent of ACCs are functional and may secrete cortisol (most commonly), aldosterone, or sex hormones [10, 13]. Some produce multiple steroid hormones [40].

On gross pathologic examination, ACCs are large and complex neoplasms with an average size of approximately 12 cm (usually ranging from 2 to 25 cm), commonly with irregular margins, cystic appearance, hemorrhage, and calcifications in 30% of cases [10, 39, 63, 64]. Although radiologic evaluation of adrenal lesions is not usually definitively diagnostic of ACC, it identifies suspicious features that raise concern for ACC. The presence of metastatic disease along with a suspicious mass is definitive of malignancy [64]. Any adrenal mass with concerning radiographic findings or size ≥ 4 cm should be resected due to increased risk of ACC [5, 8]. Imaging features suspicious of malignancy include irregular margins, intratumoral necrosis or hemorrhage, heterogeneous enhancement, invasion into adjacent structures, venous extension, and calcifications (Fig. 6.6) [64]. Some tumors may contain areas of intracellular fat, which has been attributed to the presence of cortisol and related fatty precursors in hormonally

active tumors [65]. Surgery is the mainstay of treatment for ACC and the only chance for cure of this aggressive cancer.

The appearance of ACC on UCT is a heterogeneous mass with necrosis, an attenuation value >10 HU, and displacement or invasion of adjacent structures (see Fig. 6.6) [39]. Necrosis corresponds to areas of low attenuation on UCT and is universally present in tumors >6 cm; however, smaller tumors may appear homogeneous on UCT [64]. On CECT, ACC has heterogeneous enhancement with peripheral predominance and a slower contrast medium washout than adenomas (APW $<60\%$, RPW $<40\%$) [39]. The periphery usually enhances more than the center due to central tumor necrosis. Other signs of malignancy include tumor thrombus in the renal vein and inferior vena cava (IVC), regional and para-aortic lymphadenopathy, and distant metastasis [39]. CT is also valuable in identifying metastases, which are frequently present in regional and para-aortic lymph nodes (25–46%), lungs (45–97%), liver (48–96%), and bone (11–33%) at presentation [64]. Tumor invasion into the IVC has been reported in 9–19% of ACC cases at presentation [66].

MRI is considered superior to CT for the evaluation of invasion into adjacent structures and venous involvement [13, 63, 64]. On T1w and T2w MRI, ACC appears heterogeneous because of hemorrhage and necrosis [39]. On T1w MRI,

ACC is usually iso- to slightly hypointense to liver, while on T2w MRI it is usually hyperintense to liver with heterogeneous signal in areas of hemorrhage and necrosis. Areas of hemorrhage or intracytoplasmic fat within ACCs can appear as high T1 signal intensity or signal loss on out-of-phase chemical shift imaging [39, 62, 64]. Due to the increased metabolic activity in ACC compared to benign lesions, PET imaging will show high 18-FDG uptake with a cutoff value >1.45 for adrenal-to-liver maximum SUV [67]. [FDG] PET combined with CECT has a sensitivity of 100% and a specificity of 87–97% for identifying malignant adrenal lesions. False-positive results are due to adenomas that mimic malignancy [64]. A newer PET tracer, ¹¹C-metomidate, identifies lesions of adrenocortical origin with high tracer uptake, of which ACC has the highest uptake [39, 64]. This marker allows for the differentiation of adrenal cortical lesions from pheochromocytoma and metastases, which are uptake negative [64]. The most important contribution of PET scans is the evaluation for metastatic disease, which is present in a third of patients with ACC at presentation [64]. Imaging is also critical in follow-up, as it is more sensitive than hormone surveillance [63].

Metastases

The adrenal glands are well vascularized and are a common site of metastasis from primary tumors of the lung, skin (melanoma), kidney, colon, breast, pancreas, esophagus, liver, and stomach [10, 39, 68]. Autopsy studies in patients with malignant epithelial tumors have demonstrated metastases to the adrenal glands in up to 27% of patients [40]. In a study of 2005 patients, 2.5% of AIs were found to be metastatic lesions [5]. However, in patients with a history of malignancy, approximately half of adrenal lesions turn out to be metastases [69]. Metastases to the adrenal gland are often bilateral [5, 39]. When detected early, metastases are small, homogeneous, and similar in appearance to adenomas, making it difficult to differentiate them from a nonfunctioning adrenal adenoma and other adrenal lesions on CT or MRI [2, 10]. Metastases

from renal cell carcinoma and hepatocellular carcinoma can have contrast enhancement patterns similar to benign adenomas, making it difficult to differentiate them from an adenoma [70].

Larger metastatic lesions to the adrenal glands are heterogeneous in appearance with areas of necrosis and irregular margins. On UCT, they usually measure greater than 10 HU and show delayed washout with APW <60% and RPW <40% on CECT [10]. On T1w MRI, most metastases are isointense to hypointense, while they are hyperintense on T2w MRI and have a lack of signal loss on opposed-phase CSI-MRI [10].

[FDG] PET is more useful in identifying metastases than other conventional imaging studies [FDG]. PET has a high sensitivity and specificity for identifying metastatic disease in patients with history of malignancy, demonstrating lesions with FDG-avid uptake and increased activity compared to the liver [10, 39]. False-positive and false-negative results occur on PET imaging because 16% of benign adrenal lesions are PET-avid and malignant lesions are sometimes not seen on PET scans because of hemorrhage, necrosis, and nodule size <1 cm in size [39]. Furthermore, a PET scan is not able to differentiate between a metastatic lesion and ACC [8]. Overall, PET is 97% sensitive and 91% specific in separating benign from malignant adrenal lesions [30]. When using PET/CT for characterizing adrenal masses in patients with a known malignancy, the sensitivity and specificity to detect malignancy increases to 100 and 99%, respectively, making it the most accurate method of identifying metastases to the adrenal glands [31]. FNA biopsy can be used to confirm the diagnosis of metastatic disease, which can differentiate between adrenal and nonadrenal tissue. FNA is useful in patients with a history of malignancy that have an adrenal mass <4 cm suspicious for a metastasis but difficult to delineate from a benign adrenal lesion. The reported complication rate for FNA of an adrenal mass is 2.8% and includes hematoma, abscess, abdominal pain, hematuria, pancreatitis, pneumothorax, and tumor recurrence in the biopsy tract [5, 8, 71]. Biochemical evaluation to rule out pheochromocytoma must be done prior to FNA to prevent the risk of fatal hypertensive crisis [72, 73].

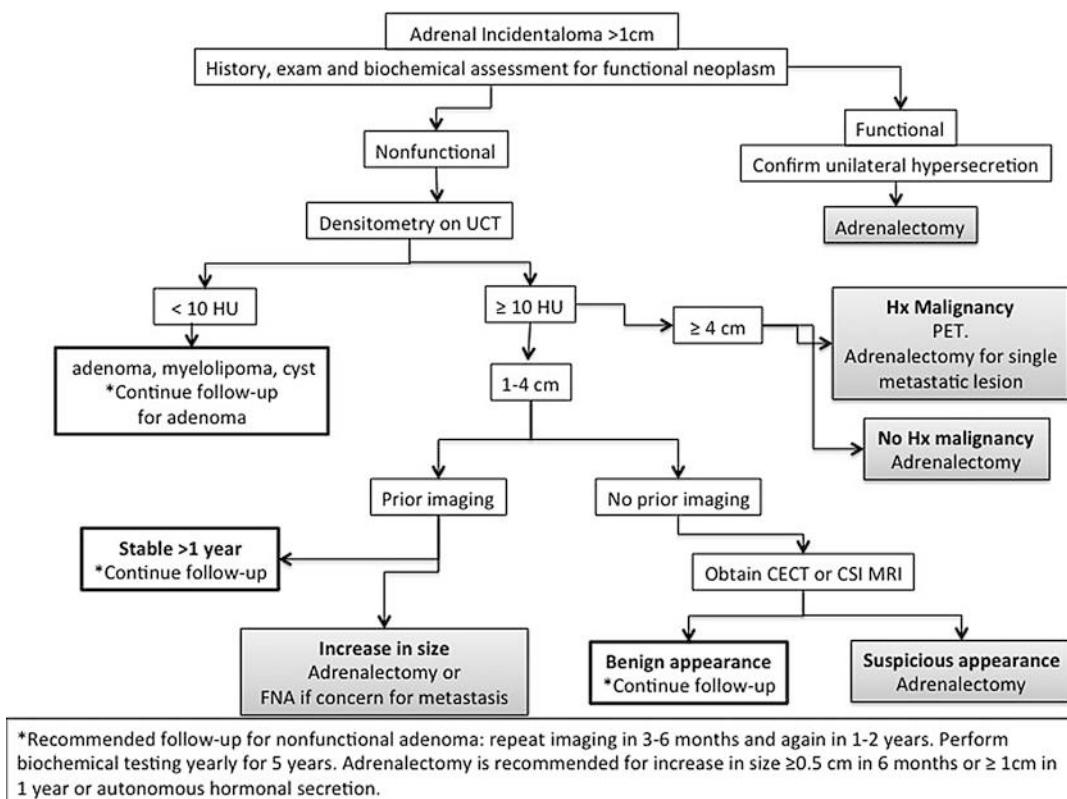


Fig. 6.7 Suggested algorithm for the assessment of an incidental adrenal lesion. (CECT contrast-enhanced CT, CSI MRI chemical shift magnetic resonance imaging, Dx diagnosis, FNA fine needle aspiration, Hx history, HU Hounsfield units, PE physical exam, PET positron emission tomography, UCT unenhanced CT)

assessment of an incidental adrenal lesion is illustrated in Fig. 6.7.

Conclusion

Adrenal lesions are increasingly being detected on cross-sectional imaging. Modern imaging techniques allow for accurate characterization of adrenal lesions without the need for a tissue diagnosis. The majority of adrenal lesions can be characterized sufficiently using a single imaging modality, multiphase CT, MRI with CSI, or [FDG] PET-CT. History, physical examination, and evaluation of the functional status of an adrenal lesion are essential. In rare cases, more than one imaging modality, AVS or percutaneous biopsy can aid in diagnosis and patient management decisions. When surgical resection is not required, follow-up imaging at 3–6 months and then annually for 1–2 years is recommended, with repeat hormonal evaluation on an annual basis for 5 years. A suggested algorithm for the

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Imaging Modalities for Pheochromocytoma and Paraganglioma

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Tumor Origin

Pheochromocytoma (PHEO) and sympathetic-associated paragangliomas (symp-PGL) develop from cells of the adrenal medulla or extra-adrenal chromaffin cells, respectively. Embryogenesis of chromaffin cells has received considerable attention over the last century. Evidence for the origin of adrenal medulla and sympathetic neurons was pursued by a number of research groups, but it was largely the work of Le Douarin and colleagues using quail/chick chimeras that led to the resolution of their neural crest (NC) origin [1]. Later,

immunohistochemical studies, in situ hybridization, transgenic animal studies, and single cell electroporation methods using fluorescent NC progenitors, significantly added to the knowledge of the required mechanisms for correct specification, migration, and differentiation of the sympathoadrenal lineage [2]. Chromaffin cells and sympathetic neurons derive from a common sympathoadrenal (SA) progenitor cell. SA progenitor cells aggregate at the dorsal aorta, where they acquire a catecholaminergic neural fate. Subsequently, the cells migrate ventrally to invade the fetal adrenal cortex and form the adrenal medulla as well as dorsolaterally to form sympathetic ganglia. Most extra-adrenal chromaffin cells regress via apoptosis. The organ of Zuckerkandl (OZ) constitutes the largest chromaffin paranganglia in the embryo and regresses after birth via autophagy [3]. Adrenal medulla and persistent extra-adrenal chromaffin cells located in the retroperitoneum and posterior mediastinum represent the chromaffin paranganglia system in adults. These embryological bases explain why PHEO and symp-PGL can be widely distributed throughout the body.

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Spectrum of Hereditary Syndromes

Research in molecular genetics has resulted in the identification of 18 susceptibility genes for tumors of the entire paranganglia system. Most PHEOs occur sporadically (>90 %) whereas the

majority of symp-PGLs are associated with germline driver mutations. Depending on their locations, the most commonly found gene mutations are as follows: (1) unilateral PHEO: succinate dehydrogenase complex subunit B (*SDHB*), von Hippel–Lindau tumor suppressor (*VHL*); (2) bilateral PHEO: *SDHB*, Ret Proto-Oncogene (*RET*), *VHL*, neurofibromin 1 (*NF1*), MYC associated factor X (*MAX*), transmembrane protein 127 (*TMEM127*); (3) symp-PGLs with or without PHEO: *SDHB*, succinate dehydrogenase complex subunit D (*SDHD*), *VHL*, hypoxia-inducible factor 2-alpha, also called EPAS1 (*HIF2A*). Other genes account for a small minority of cases. Tumor sequencing has also led to the identification of somatic events in a large number of PHEOs and PGLs.

Clinical Presentation

PHEO/PGLs are rare tumors (annual incidence of 0.1–0.6 per 100,000 population). They account for about 4% of adrenal incidentalomas with a higher prevalence in autopsy series. PHEOs and symp-PGLs usually cause symptoms of catecholamine oversecretion (e.g., sustained or paroxysmal elevations in blood pressure, headache, episodic profuse sweating, palpitations, pallor, and apprehension or anxiety).

Anatomic Imaging

Computed Tomography (CT)

Patient Preparation and Imaging Protocol

At our institution, patients are asked to fast for 2 h [4] prior to the study. To diagnose PHEO, we use a number of CT protocols. The “adrenal nodule protocol” consists of three series—noncontrast, contrast enhanced (at 60 s), and delayed images (at 15 min) of the abdomen. The attenuation of the adrenal nodule is measured on the three series and washout characteristics calculated [4]. In cases where the location of a PHEO is unclear but is clinically or biochemically suspected, a noncon-

trast CT is ordered. If later deemed necessary, a contrast-enhanced (at 60 s) CT is obtained of the chest, abdomen, and pelvis. Depending on body weight, (80 cc if <180 pounds, 100 cc if <280 pounds, 120 cc if >280 pounds), Isovue 370 is administered. Previously, there have been concerns regarding the administration of iodinated contrast to patients with PHEO. However, we now normally administer contrast. A retrospective review of patients who received nonionic contrast demonstrated the safety of intravenous contrast, even in patients who had not received alpha or beta blockade [5]. A small, prospective study of patients receiving low-osmolar contrast media also demonstrated safety [6]. An occasionally employed, alternative practice is to discontinue the protocol after the noncontrast scan if an adrenal mass or a mass along the sympathetic chain is discovered in a patient suspected of having a PHEO. If considered necessary for diagnosis or surgical planning, we follow with iodinated contrast or an MRI scan, the latter of which would avoid the use of iodinated contrast entirely.

Previously, our studies were performed at 120 kV. We now perform weight-based protocols and in patients less than 200 pounds, we use a lower tube voltage of 100 kV with a usual mA of 300.

Normal Appearances and Abnormalities

The adrenal glands are a pair of retroperitoneal, thin, inverted, V or Y shaped organs with flat or concave margins [7]. The vertical length can range from 2 to 4 cm. Both limbs measure approximately 4 mm in cross-section [8].

On noncontrast CT, a PHEO can demonstrate a variety of appearances. They can be low density or of soft tissue attenuation. Two-thirds of PHEO are solid, while the remainder are complex or have undergone cystic change [9]. Reliably differentiating an adenoma from a PHEO can be problematic. Typically, the CT attenuation of PHEO is of soft tissue attenuation and thus, greater than 10 Hounsfield units (HU). However, in the rare circumstance where a PHEO contains fat, attenuation values can be similar to adenomas, measuring less than 10 HU [10]. PHEO can

be of high attenuation due to the presence of hemorrhage [11]. Although infrequent, calcifications may be present in approximately 10% of cases [12]. PHEO typically demonstrate avid enhancement [13] and any adrenal lesion that enhances greater than 130 HU on multidetector CT cannot be assumed to be an adenoma [14]. In addition, enhancement can be heterogeneous or there may be no enhancement due to cystic or degenerated regions within the lesion. In terms of washout pattern, PHEO can demonstrate varying and nonuniform washout patterns, leading to the inability to reliably differentiate PHEO from adenomas and metastasis to the adrenal gland [10, 15, 16]. A substantial minority of PHEO (33%), especially when hypervascular have washout levels similar to those of adenomas [17].

Diagnostic Accuracy

To detect PHEO in the adrenal gland, the sensitivity of CT ranges from 76 to 100% [18–21]. Specificity is much lower in distinguishing PHEO, adrenal adenomas, and myelolipomas with figures as low as 50% [18, 20, 21]. Overall sensitivity of CT for the detection of extra-adrenal PHEO as well as recurrent, residual, or metastatic tumors can be as low as 57% [20, 22–24]. Specificity is also low with reported values of 50% [25].

Magnetic Resonance Imaging (MRI)

Patient Preparation and Imaging Protocol

At the time of referral, the clinician is responsible for documenting that the patient does not have any contraindications to undergoing MRI. The patient is also asked to verify this on the day of the study.

In patients with suspected PHEO, our MR imaging protocol includes a coronal, breath-hold T2 HASTE; axial, gradient-recalled echo T1 chemical-shift imaging with in- and out-of-phase breath-hold images; axial fast spin-echo T2-weighted fat-saturated or long TE inversion recovery breath-hold images, as well as axial precontrast and dynamic-enhanced gradient-

recalled-echo 3D volumetric interpolated breath-hold examination images. For an abdominal MRI, anatomic coverage should extend from the diaphragm to the aortic bifurcation. In order to also detect extra-adrenal PGLs along the lower sympathetic chain, a pelvic MRI can be added if complete coverage is desired.

As MRI does not confer any ionizing radiation, there is no radiation dose to the patient. This is particularly advantageous in young patients or those who need to undergo repeat imaging.

Normal Appearances and Abnormalities

The normal adrenal gland demonstrates low to intermediate signal on T1- and T2-weighted imaging [26]. The classic imaging appearance of PHEO is “light-bulb” bright on T2-weighted imaging. In reality, the prevalence of this appearance is low with reported ranges between 1 and 65% of PHEO [27–29] with lowest figures in studies with current technology MRIs. Thirty percent of PHEO demonstrate moderate or low T2-weighted signal intensity [13, 30]. A PHEO is usually hypointense on T1-weighted imaging [13], although the presence of fat or hemorrhaging could lead to high signal intensity on T1. However, PHEO do not usually contain fat and thus, maintain their signal on opposed-phase gradient-echo images [31]. On rare occasions, they can contain microscopic fat leading to signal loss on chemical shift [13]. PHEO typically demonstrate avid contrast enhancement following the administration of intravenous gadolinium-based contrast material [28, 32], similar to enhancement on CT (Figs. 7.1, 7.2, and 7.3). Diffusion-weighted MRI may provide useful additional information for the diagnosis of PHEO. PHEOs have been demonstrated to have relatively higher Apparent Diffusion Coefficient (ADC) values than adrenocortical adenomas and malignancies [33]. MRI spectroscopy is an emerging technique that has shown promise in small cohort studies [34]. A unique spectral signature of PHEO not seen in adenomas was demonstrated using a 2D PACE single-voxel MR spectroscopy sequence [34]. Further evaluation in a larger series will be necessary for validation of this technique.

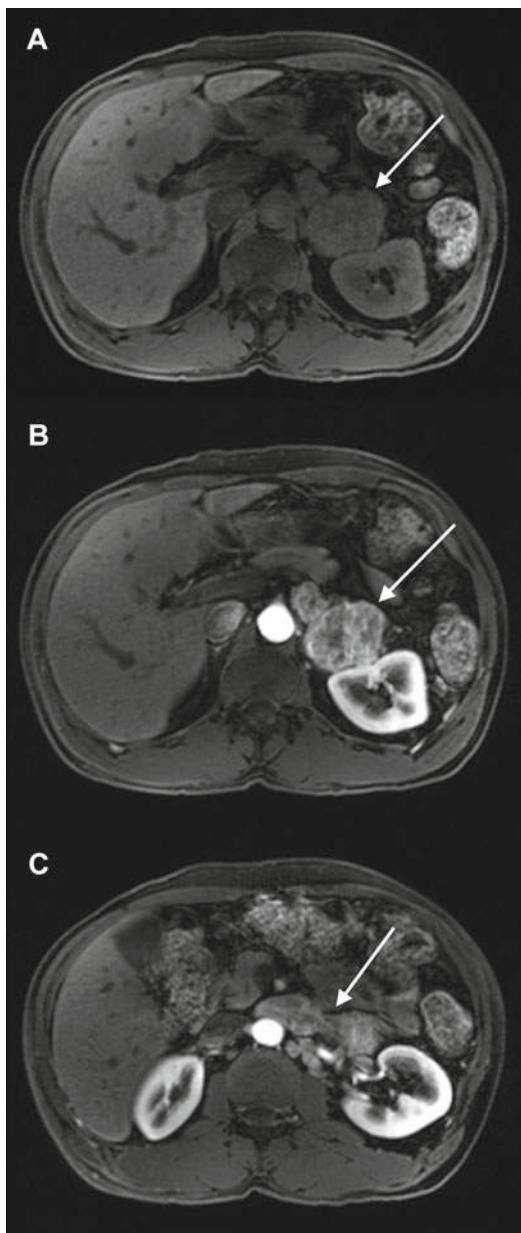


Fig. 7.1 Fifty-two-year-old man with hypertension. Precontrast, axial T1-weighted image with fat saturation (a), demonstrates a large left adrenal mass (arrow). On the postcontrast axial T1-weighted image with fat saturation (b), there is heterogeneous enhancement (arrow). (c) (postcontrast axial T1 weighted image with fat saturation) demonstrates invasion of the left renal vein (arrow). This was a pathologically confirmed pheochromocytoma

Diagnostic Accuracy

For detecting PHEO in the adrenal gland, the sensitivity of MRI ranges between 91 and 100 % with a specificity of approximately 50–97 % [18,

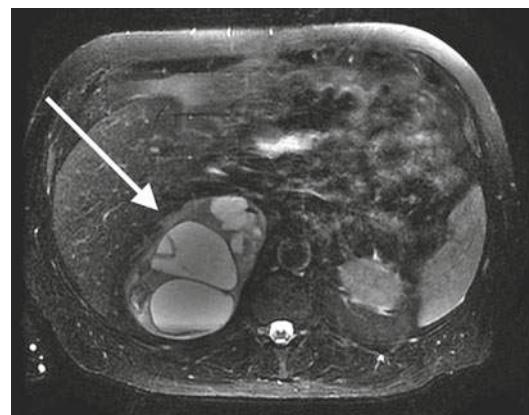


Fig. 7.2 Seventy-year-old man with incidentally discovered right adrenal mass. Axial, fast spin echo, fat saturated sequence demonstrating an enlarged, heterogeneous right adrenal mass (arrow) with hemorrhagic cystic areas within and internal septations. This was confirmed on resection to demonstrate a pheochromocytoma

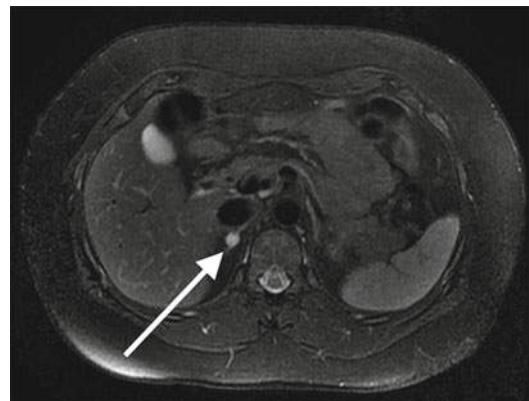


Fig. 7.3 Twenty-six-year-old woman with von Hippel-Lindau syndrome. Axial T2-weighted image demonstrates a small, T2 bright mass (arrow) within the right adrenal gland. This was a pathologically proven pheochromocytoma. This patient had previous resection of the left adrenal gland, which was also a pathologically confirmed pheochromocytoma

19, 23, 28, 35]. For the detection of adrenal and extra-adrenal PGLs, the sensitivity is 93 % with a specificity ranging from 50 to 100 % [25, 36]. MRI has been reported as superior to CT for the detection of extra-adrenal tumors [21, 37, 38].

Malignant PHEO

On imaging, it is difficult to diagnose malignant PHEO in the absence of metastatic disease [12]. The presence of vascular or capsular invasion is

not a pathognomonic feature of malignancy, although it is identified more commonly in malignant, rather than benign PHEO [39]. Diffusion-weighted MR imaging can be advantageous in depicting lymph node and liver metastases and may have a higher rate of detecting metastatic lesions compared with Metaiodobenzylguanidine scintigraphy or FDG-PET [40].

Radionuclide Imaging and Metabotypes

The major advantage of nuclear imaging is in providing a high visual contrast between tumor and healthy tissue, which enables the detection of tumors that could potentially be missed by conventional imaging. Beyond its localization value, this imaging modality provides unique opportunities for enhanced characterization of these tumors at the molecular level (e.g., catecholamine synthesis, transporter expression, somatostatin receptor expression, glucose metabolism), mirroring *ex vivo* histologic classification, but on a whole-body, *in vivo*, scale [41]. This opportunity is currently possible with a number of excellent radiopharmaceuticals, which target different functional and molecular pathways that often reflect the diverse genetic landscape of PHEO/PGL.

¹²³I-Metaiodobenzylguanidine (MIBG) and Catecholaminergic Phenotype

Cellular Uptake and Interference

MIBG is an iodinated analog of guanidine, which is structurally similar to norepinephrine. It is taken up by cells via the norepinephrine transporter and stored within the neurosecretory granules via vesicular monoamine transporters 1 and 2.

Many drugs modify the uptake and storage of MIBG such as opioids, tricyclic antidepressants, sympathomimetics, antipsychotics, and some antihypertensive agents such as Labetalol.

Patient Preparation, Imaging Protocol, and Dosimetry

Thyroid blockade is started a day before tracer injection and continued for 2 days after a ¹²³I-MIBG scan and 5 days for a ¹³¹I-MIBG scan. Discontinuation of drugs interfering with MIBG uptake and retention is required. All medications must be withheld for 1–3 days prior to imaging, with the exception of labetalol and depot forms of antipsychotics, for which the suggested withdrawal period is 1 month.

¹²³I-MIBG scintigraphy is preferable to ¹³¹I-MIBG scintigraphy because it provides higher quality images and lower radiation exposure. ¹²³I-MIBG scans are usually obtained 24 h after tracer injection (200–400 MBq) and consist of planar static images and in adults, SPECT/CT over the anatomical regions showing pathological tracer uptake.

The effective dose for ¹²³I-MIBG is 0.013 mSv/MBq. The radiation dose is higher when CT is used in SPECT/CT protocols.

Normal Distribution and Abnormal Patterns

Normal uptake of ¹²³I-MIBG can be observed in the myocardium, salivary glands, thyroid gland (if no adequate thyroid blockade is performed), liver, lungs, adrenal glands, and bowel. The large intestine may also be visible. Brown adipose tissue uptake should be considered within the normal distribution of MIBG, although this may be more common in children than adults. ¹²³I-MIBG uptake in the adrenal glands is considered normal if mild (less or equal to liver uptake), symmetric, and when the glands are not enlarged on CT.

High intensity adrenal uptake (more intense than the liver) or inhomogeneous adrenal uptake on the side of the abnormal adrenal on CT is considered abnormal. Extra-adrenal sites of uptake that cannot be explained by normal physiological distribution are considered abnormal.

Diagnostic Accuracy

PHEO and symp-PGL exhibit a catecholaminergic phenotype on imaging. ¹²³I-MIBG scintigraphy has a sensitivity ranging from 83 to 100% and a very

high specificity (98–100 %) in detecting primary tumors. Its sensitivity is lower in small tumors, SDHx-related PHEO/PGL, metastatic PHEO/PGL, and head and neck PGL [42–48].

¹⁸F-Fluorodopa (¹⁸F-DOPA) and Amino Acid Uptake Phenotype

Cellular Uptake and Interference

Dihydroxyphenylalanine is the precursor of catecholamines. ¹⁸F-DOPA is taken up through neutral amino acid transporters (mainly LAT-1 and 2) and decarboxylated into ¹⁸F-Dopamine by cytosolic aromatic L-amino acid decarboxylase (AADC). There is no reported drug interaction in PHEO and PGL.

Patient Preparation, Imaging Protocol, and Dosimetry

Patients should fast for at least 3 h prior to injection. The administration of 200 mg of carbidopa 1–2 h prior to ¹⁸F-DOPA injection has been reported to increase tumor uptake [49]. Scans are usually obtained 30–60 min after tracer injection from the base of the skull to mid-thighs (or over the whole body depending on the clinical setting). Additional early acquisition (at 10 min after tracer injection) centered over the abdomen can be performed to overcome difficulties in localizing abdominal PGL located near the hepatobiliary system due to physiological tracer elimination. The effective dose equivalent in adults range from 0.0199 to 0.0539 mSv/MBq with a higher radiation dose when combined with CT imaging.

Normal Distribution and Abnormal Patterns

Physiological distribution includes the striatum, kidneys, ureter, bladder, pancreas, liver, gallbladder, biliary tract, and duodenum. Adrenal glands are faintly visible.

Any nonphysiological extra-adrenal focal uptake, asymmetrical adrenal uptake with concordant enlarged gland, or adrenal uptake more intense than liver with concordant enlarged gland, should be considered abnormal.

Diagnostic Accuracy

PHEO and symp-PGL exhibit an amino acid uptake phenotype. ¹⁸F-DOPA PET/CT has a sensitivity approaching 100 % for PHEO and a very high specificity (95 %) for symp-PGL. Sensitivity is lower in SDHx-PHEO/symp-PGL.

⁶⁸Ga DOTA-Coupled Somatostatin Agonists and Somatostatin Receptor Expression Phenotype

Cellular Uptake and Interference

The DOTA-coupled somatostatin agonists bind to somatostatin receptors (SST) and induce rapid internalization of the ligand/receptor complex. The 3 currently available agonists have an excellent affinity for SST2: DOTATOC (Tyr3-octreotide), DOTATATE (Tyr3-octreotate), and DOTANOC (Nal3-octreotide). DOTATATE has an approximately tenfold higher affinity than DOTATOC and DOTANOC for SST2. DOTANOC binds specifically to SST5, although PHEO/PGL tumors express this subtype in only a small minority of cases.

Patient Preparation, Imaging Protocol, and Dosimetry

⁶⁸Ga-conjugated peptides are available as “homemade radiotracers” with an extemporaneous preparation. There is no need for fasting before injection. It has been recommended to discontinue octreotide therapy (1 day for short-lived molecules and 3–4 weeks for long-acting analogs). Scans are usually obtained 45–90 min after tracer injection from the base of the skull to mid-thighs (or over the whole body depending on the clinical setting).

The effective dose ranges from 0.0042 to 0.015 µSv/MBq with higher radiation dose when CT used.

Normal Distribution and Abnormal Patterns

Intense physiological accumulation of radioactivity is seen in the spleen (and accessory spleen if present), kidneys, ureter, bladder, adrenals, salivary glands, and pituitary. Accumulation in

the liver is usually less intense than noted in the spleen. The thyroid can be faintly visible. Additionally, variable tracer uptake is frequently found in the pancreas, particularly in the uncinate process. Prostate gland and breast glandular tissue may show diffuse, low-grade uptake.

Diagnostic Accuracy

PHEO and symp-PGL almost always exhibit a somatostatin receptor expression phenotype. ⁶⁸Ga-DOTA-SSA was found to be more sensitive to other tracers in metastatic PHEO/PGL and head and neck PGL (lesion-based detection rate approaching 100%) [50–52]. It has been less studied in the context of nonmetastatic PHEO/ sympathoadrenal paraganglioma than in other hereditary cases, such as VHL. In one study, the sensitivity of ⁶⁸Ga-DOTATATE was 80% in sporadic PHEO [53]. ⁶⁸Ga-DOTA-SSA PET imaging can be falsely positive in metastatic lymph nodes due to various cancers, meningiomas, and other central nervous, inflammatory processes, and rare conditions such as fibrous dysplasia [53]. Nevertheless, this is often not a serious issue, since head and neck PGLs have a specific location and exhibit highly elevated uptake values [54].

¹⁸F-FDG PET and Glucose Metabolism Phenotype

Cellular Uptake and Interference

¹⁸F-FDG is taken up by tumor cells via glucose membrane transporters and phosphorylated by hexokinase into ¹⁸F-FDG-6P. ¹⁸F-FDG-6P does not follow further enzymatic pathways and accumulates proportionally to the glycolytic cellular rate. It is remarkable that tumors associated with TCA defect (*SDHx* mutations) exhibit an increased ¹⁸F-FDG uptake.

Patient Preparation, Imaging Protocol, and Dosimetry

Patients must fast for at least 6 h. PHEO patients with secondary diabetes require specific instructions for glucose control. Scans are usually obtained at 60 min (45–90 min)

postinjection. The effective dose equivalent is 2×10^{-2} mSv/MBq.

Normal Distribution and Abnormal Patterns

Physiological distribution includes brain cortex, salivary glands, lymphatic tissue of the Waldeyer's ring, muscles, brown fat, myocardium, liver, kidneys and bladder, gastrointestinal tract, testis, uterus, and ovaries (before menopause). Physiologic ¹⁸F-FDG uptake in brown adipose tissue (BAT) occurs predominantly in norepinephrine secreting PHEO/PGL.

Any nonphysiological, extra-adrenal focal uptake or adrenal uptake more intense than the liver with concordant enlarged gland should be considered abnormal.

Diagnostic Accuracy

PHEO and symp-PGL may exhibit a glucose metabolism phenotype. ¹⁸F-FDG PET uptake pattern is mainly influenced by tumor location and genetic status of patients with highly elevated uptake values in SDHx-related metastatic and nonmetastatic PHEO/PGL [55–57]. ¹⁸F-FDG PET positivity is present in about 80% of primary PHEO. Several potential diagnoses should be considered in cases of ¹⁸F-FDG-avid adrenal masses.

Other Tracers

Other tracers such as ^{99m}Tc-hydrazinonicotinamide-Tyr(3)-octreotide, ¹⁸F-FDA (fluorodopamine) PET, and ¹¹C-hydroxyephedrine (¹¹C-HED) are currently used, although they are less common.

Head-to-Head Comparison Between Radiopharmaceuticals

¹⁸F-FDOPA or ⁶⁸Ga-DOTA-SSA vs. ¹²³I-MIBG

¹⁸F-FDOPA [44] and ⁶⁸Ga-DOTA-SSA [58–60] were found to be superior to ^{123/131}I-MIBG in metastatic/multifocal cases of PHEO/PGL.

⁶⁸Ga-DOTA-SSA vs. ¹⁸F-FDG

⁶⁸Ga-DOTATATE was compared to ¹⁸F-FDG in a series of 17 metastatic *SDHB*-related PHEO/PGL and identified more lesions than ¹⁸F-FDG (lesion-based detection rate of 98.6 % vs. 85.8 %) [52].

⁶⁸Ga-DOTATATE was also found to be superior to ¹⁸F-FDG in sporadic metastatic cases [51] and in head and neck PGL.

⁶⁸Ga-DOTA-SSA vs. ¹⁸F-FDOPA

The use of ⁶⁸Ga-DOTA-SSA in the context of PHEO/PGLs has been less studied than gastroenteropancreatic neuroendocrine tumors. A head-to-head comparison between ⁶⁸Ga-DOTA-SSA and ¹⁸F-FDOPA PET has been performed in only five studies: one retrospective study from Innsbruck Medical University (⁶⁸Ga-DOTATOC in 20 patients with unknown genetic background) [61], 3 prospective studies from the National Institutes of Health (*SDHB*, head and neck PGL, and sporadic metastatic disease) (⁶⁸Ga-DOTATATE in 17 and 20 patients) [50–52], and one prospective study from La Timone university hospital (⁶⁸Ga-DOTATATE in 30 patients). In these studies, ⁶⁸Ga-DOTA-SSA PET/CT detected more primary head and neck PGLs as well as *SDHx*-associated PGLs than ¹⁸F-FDOPA PET/CT [53]. By contrast, in the context of sporadic PHEO, ¹⁸F-FDOPA PET/CT may detect more lesions than ⁶⁸Ga-DOTATATE [53]. One of the main drawbacks of ⁶⁸Ga-DOTA-SSA is the very high physiological uptake by healthy adrenal glands [62].

Current Role of Imaging for PHEO/ PGLs

Successful PHEO/PGL management requires an interdisciplinary team approach. Precise identification of clinical context and genetic status of patients enables a personalized use of functional imaging modalities. Currently, it is recommended to adopt a tailored approach using a diagnostic algorithm based on tumor location, biochemical phenotype, and any known genetic background (Table 7.1) [41, 63]. However, it should be underlined that selection of the appropriate imaging pathway using algorithms is, itself, somewhat

challenging, because it requires information that is not always readily available at the time of investigating a suspected PHEO/PGL.

Diagnosis of PHEO/Symp-PGL

Adrenal Mass

The diagnosis of PHEO relies on the identification of excessive secretion of metanephrenes. Functional imaging should be used in a small minority of cases such as those with suspicion of nonfunctioning PHEO on CT/MRI, mild elevation of metanephrenes in the presence of an adrenal mass, acute cardiovascular complication in the critical care setting, hemorrhagic adrenal masses, and elevated metanephrenes in renal insufficiency. Catecholamines can also be detected in vivo by proton single-voxel Magnetic Resonance Spectroscopy (¹H-MRS). PET imaging using ¹⁸F-FDOPA PET or ⁶⁸Ga-DOTA-SSA is highly sensitive.

Retroperitoneal Extra-adrenal Nonrenal Mass

In the presence of a retroperitoneal extra-adrenal nonrenal mass, it is important to differentiate a PGL from other tumors or lymph node involvement, including metastases. A biopsy is not always contributory or even recommended since it can carry a high risk of hypertensive crisis and tachyarrhythmia and therefore, should only be done if PHEO/PGL is ruled out in any patient presenting with signs and symptoms of catecholamine excess. Specific functional imaging studies, which are not usually performed before biochemical results are available, are very helpful in distinguishing PGLs from other tumors. ⁶⁸Ga-DOTA-SSA is the first-line imaging since most patients are expected to have *SDHx* mutations.

Diagnosis of Malignancy

Presently, there are no reliable cytological, histological, immunohistochemical, molecular, or imaging criteria for determining malignancy [64].

Table 7.1 Stepwise molecular imaging approaches for pheochromocytoma/paraganglioma (PGL)

Localization	Gene	First line	Second line
PHEO	<i>MEN2 (RET), SDHx, VHL, NF1, TMEM127, MAX</i>	¹⁸ F-FDOPA	⁶⁸ Ga-DOTATATE
Symp-PGL	<i>VHL, SDHx, Carney triad, HIF2A, PHD1/2</i>	⁶⁸ Ga-DOTATATE	¹⁸ F-FDOPA
Parasymp-PGL (head and neck)	<i>SDHx, SDHAF2</i>	⁶⁸ Ga-DOTATATE	¹⁸ F-FDOPA
Metastatic PPGL	<i>SDHx (B > D), FH</i>	⁶⁸ Ga-DOTATATE	¹⁸ F-FDG in SDHx ¹⁸ F-FDOPA in sporadic

PHEO pheochromocytoma, *Symp PGL* sympathetic-associated paragangliomas, *Parasymp-PGL* parasympathetic-associated paragangliomas

The diagnosis of malignancy remains strictly based on the finding of metastases where chromaffin cells are not usually present, such as the lymph nodes, lung, bone, or liver.

Anatomical imaging appears sufficient for localizing PHEO. Functional imaging is probably not necessary in the preoperative workup of patients meeting the following criteria: >40 years, no family history, small (<3.0 cm) PHEO secreting predominantly metanephrine, and negative genetic testing. Functional imaging is strongly recommended for excluding metastatic disease in large adrenal tumors (>6.0 cm) and in *SDHB* patients. It is widely accepted that tumors with an underlying *SDHB* mutation are associated with a higher risk of aggressive behavior, development of metastatic disease, and ultimately, death. In these patients, there is a clear advantage of using ⁶⁸Ga-DOTA-SSA over ¹²³I-MIBG SPECT and even ¹⁸F-FDG in the presence of *SDHB* mutation.

Staging

Inherited and Symp-PGL

Beyond malignancy risk, inherited (especially *SDHx* and *VHL*) or Symp-PGL raise the problem of multifocality. Based on recent published data, it is anticipated that ⁶⁸Ga-DOTA-SSA will rapidly get a leading position in this setting. In absence of available ⁶⁸Ga-DOTA-SSA, ¹⁸F-FDG should be preferred to ¹⁸F-FDOPA in *SDHx* patients (Fig. 7.4) whereas ¹⁸F-FDOPA appears to be an excellent first-line imaging tool in other genotypes and sporadic cases (Fig. 7.5).

Metastatic Disease

Proper staging and early detection of metastatic disease is a key point for choosing the necessary treatment plan, follow-up, and outcome for these patients. CT, whole-body MRI, and PET imaging provide the most useful complementary information. The presence of *SDHx* mutations markedly influences sensitivity of ¹⁸F-FDG and ¹⁸F-FDOPA PET/CT, whereas ⁶⁸Ga-DOTA-SSA seem to have an excellent sensitivity regardless of genetic background (Table 7.2). To date, ¹⁸F-FDOPA PET or ⁶⁸Ga-DOTA-SSA may be the imaging modality of choice in the absence of a *SDHB* mutation, or when genetic status is unknown. By contrast, ⁶⁸Ga-DOTA-SSA or, if not available, ¹⁸F-FDG PET, should be considered as the imaging modalities of reference for *SDHx*-related cases (Fig. 7.6).

Image-Based Treatment of PHEOs/ PGLs

Adrenal Sparing Surgery

Subtotal (cortical-sparing) adrenalectomy is a valid option in *MEN2*, *NF1*, or *VHL*. In cases with bilateral PHEO, this strategy offers the advantage of potentially avoiding steroid supplementation. Therefore, it is crucial to perform regular imaging follow-up of known PHEOs in addition to biochemical testing for determining the optimal time to schedule cortical-sparing surgery. CT is preferable over MRI due to its excellent resolution, which provides detailed anatomic locations of tumor extension within the adrenal gland and, for *MEN2* patients, the number of tumors within the

Fig. 7.4 ^{18}F -FDOPA (a) compared to ^{18}F -FDG (b) in a case with multifocal SDHx-related pheochromocytoma/paraganglioma. The latter is clearly superior. Arrows indicate missed lesions on ^{18}F -FDOPA PET/CT

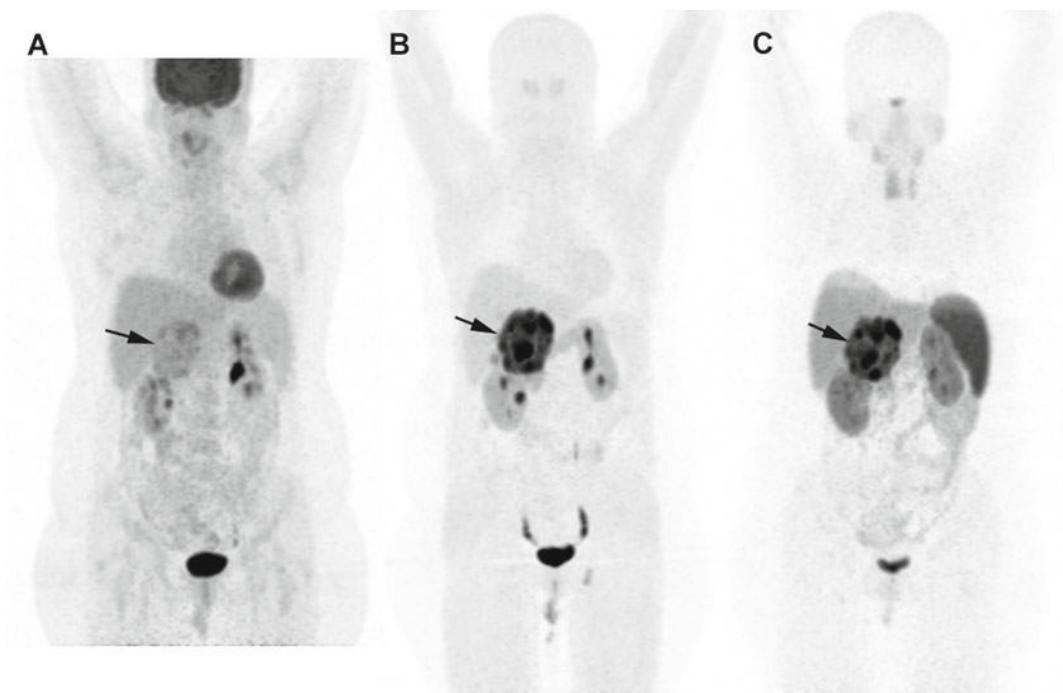
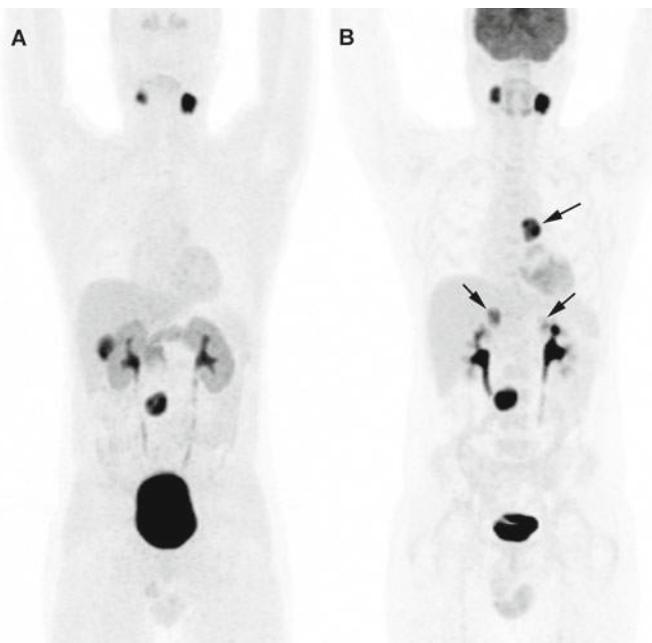


Fig. 7.5 Head-to-head comparison of ^{18}F -FDG (a), ^{18}F -FDOPA (b), and ^{68}Ga -DOTATATE (c) in patients with sporadic pheochromocytoma. See the low FDG tumor uptake compared to other radiopharmaceuticals

Table 7.2 Number of identified lesions and detection rate in ^{68}Ga -DOTATATE, ^{18}F -FDG, ^{18}F -FDOPA, ^{18}F -FDA-PET/CT, and CT/MRI in metastatic *SDHB* mutation related pheochromocytoma/paraganglioma (PHEO/PGL)

	68Ga-DOTATATE	18 F-FDG	18 F-FDOPA	18F-FDA	CT/MRI
All compartments	294/298 98.7 %	257/298 86.2 %	175/285 61.4 %	148/285 51.9 %	254/298 85.2 %
Mediastinum	65/65 100 %	57/65 87.7 %	39/65 60.0 %	39/65 60.0 %	55/65 84.6 %
Lungs	62/63 98.4 %	45/63 71.4 %	45/63 71.4 %	18/63 28.6 %	62/63 98.4 %
Abdomen	49/49 100 %	46/49 93.9 %	31/43 72.1 %	19/43 44.2 %	38/49 77.6 %
Liver	5/5 100 %	3/5 60.0 %	4/5 80.0 %	0/5 0.0 %	5/5 100 %
Bone	96/99 97.0 %	92/99 92.9 %	41/94 43.6 %	57/94 60.6 %	83/99 83.8 %

CT computed tomography, MRI magnetic resonance imaging

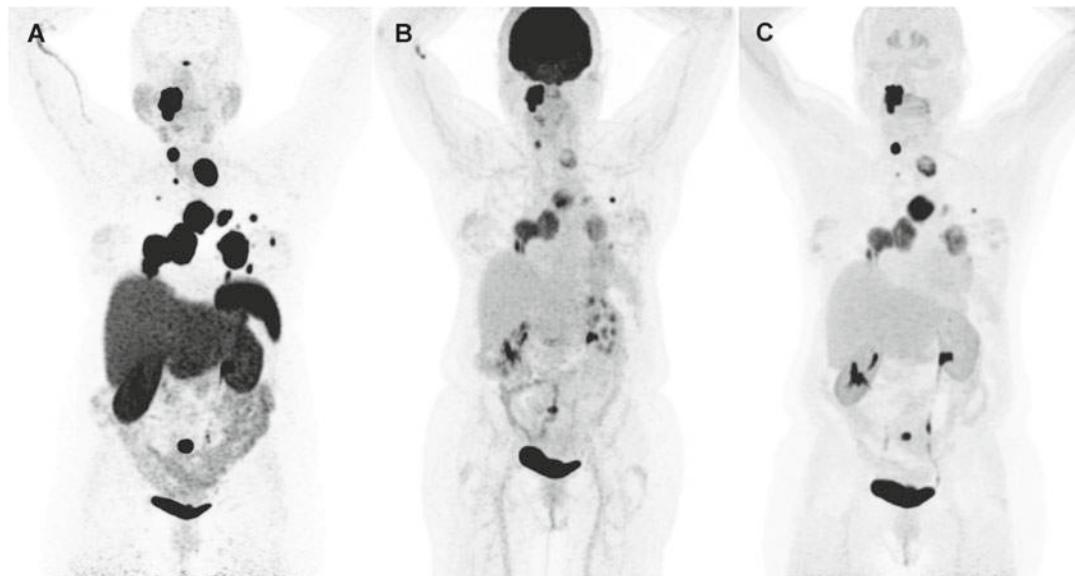


Fig. 7.6 Superiority of ^{68}Ga -DOTATATE to other molecular imaging modalities in *SDHB*-related metastatic pheochromocytoma/paraganglioma (PPGL). Metastatic PPGL with (a) ^{68}Ga -DOTATATE, (b) ^{18}F -FDG, (c) ^{18}F -FDOPA

adrenal medulla. On the other hand, the advantage of using MRI over CT is the lack of exposure to ionizing radiation, which is an important factor in hereditary cases undergoing continuous follow-up. In selected cases, functional imaging may be used in addition to anatomic imaging. There is a clear advantage of ^{18}F -FDOPA PET over MIBG and other specific PET tracers due to the lack of significant uptake in normal adrenal glands [65]. ^{18}F -FDOPA PET may also identify metastases

from medullary thyroid cancer with persistent hypercalcitoninemia.

Theranostics

^{123}I -MIBG scintigraphy is used as a companion imaging agent to assist in radionuclide therapy selection. A special advantage of labeled SSAs is that, unlike ^{18}F -FDOPA, they can be used in the radioactive treatment of these tumors (as theranostic agents). To date, peptide receptor radionuclide

therapy (PRRT) using $^{90}\text{Y}/^{177}\text{Lu}$ -labeled somatostatin agonists has been evaluated in a limited number of PHEO/PGL cases [66–68]. On average, response rates (mainly partial responses) have been 30–60%. Disease stabilization is frequent, but more difficult to interpret since these tumors often exhibit a slow growing pattern. Larger studies including various hereditary and nonhereditary PHEO/PGLs are needed in order to conclude which PHEO/PGLs can be best treated using this therapy, and whether PRRT should be used together or as a “replacement” to other treatment modalities. Recent reports have shown that cellular internalization might shorten the residual time of ^{177}Lu within tumor cells compared to radiolabeled SST antagonists. SST antagonists also have higher affinities for SST receptors than agonists, and lower internalization rates, resulting in a longer retention time on cell membrane. According to these observations, somatostatin antagonists might be considered as an alternative to agonists for PRRT sometime in the future.

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Primary Hyperaldosteronism

8

Saïd C. Azoury and Aarti Mathur

Abbreviations

ACE	Angiotensin-converting enzyme
APACC	Aldosterone-producing adrenocortical carcinoma
ARBs	Angiotensin II receptor blockers
ARR	Aldosterone-to-renin ratio
AVS	Adrenal venous sampling
BAH	Bilateral adrenal hyperplasia
CCT	Captopril challenge test
FHI-I	Familial hyperaldosteronism type I
FH-II	Familial hyperaldosteronism type II
FH-III	Familial hyperaldosteronism type III
FST	Fludrocortisone suppression test
FUT	Furosemide upright test
GRA	Glucocorticoid remediable aldosteronism
PA	Primary aldosteronism
PAC	Plasma aldosterone concentration
PRA	Plasma renin activity
SST	Saline suppression testing

Background

In 1956, Jerome Conn first described a 34-year-old female with high blood pressure, severe hypokalemia, and mild hypernatremia with an average 22-fold higher mineralocorticoid activity per day. Her clinical condition reversed after removal of a right adrenal mass [1]. Primary aldosteronism (PA) is now thought to be one of the most common forms of secondary hypertension and affects nearly 8.5 million people in the US [2, 3]. A Task Force of the Endocrine Society has defined it as a group of disorders in which aldosterone production is inappropriately elevated for sodium status, nonsuppressible by salt loading, and relatively autonomous of the major regulators of secretion [2]. The most common etiologies of PA are bilateral adrenal hyperplasia (BAH) (65–70 %) and an aldosterone-producing adenoma (30–35 %) [3–6]. Adrenalectomy for unilateral hypersecretion is curative and has been shown to reverse adverse cardiac and renal outcomes and improved quality of life [2]. Therefore, early detection and appropriate management is critical as PA is thought to be one of the most common curable reasons for secondary hypertension [7–10]. Herein, the authors provide an up-to-date comprehensive review on the clinical presentation, subtype classification, diagnosis, and management of PA.

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Epidemiology

Hyperaldosteronism typically presents in the third to sixth decades of life and can rarely occur in the pediatric population [11, 12]. Although its exact prevalence is unknown, several large population-based studies have provided estimates. The Framingham Offspring study, which consisted of the offspring of the original Framingham cohort, screened 3326 adults and found an aldosterone/plasma-renin concentration exceeding 26 ng/l per mU/l in 7.9% of untreated hypertensive men and in 23.1% of untreated hypertensive women [13]. Rossi et al. identified 11.2% of 1125 hypertensive patients who met criteria for the diagnosis of PA by measurement of plasma aldosterone, renin, saline suppression test, and a captopril test [7]. Therefore, PA is thought to be present in greater than 10% of hypertensive patients and 20% of drug-resistant hypertensive patients [14, 15]. Ethnicity and gender does not appear to affect its prevalence, although African Americans may be at a greater risk of hypertension-related morbidity [15, 16]. PA may be associated with an increasing incidence of sleep apnea syndrome and obesity as it was diagnosed in 33.9% of hypertensive patients and up to 85% in those with resistant hypertension and obstructive sleep apnea [17, 18].

Target Organ Effects of Aldosterone Excess

In addition to hypertension, aldosterone excess has many other downstream consequences independent of the direct effects of high blood pressure on tissue. In PA, production of aldosterone, a salt retaining hormone, by the adrenal cortex is excessive and autonomous from the body's sodium and volume status. Over time, the excessive retention of sodium at the distal convoluted tubule leads to development of hypertension. Potassium and hydrogen are excreted in exchange for sodium, which if prolonged and severe enough can lead to hypokalemia and metabolic alkalosis. Both hypokalemia and excess aldosterone directly affect insulin action in the pancreas and can lead

to glucose intolerance and diabetes [19]. Additionally, aldosterone excess results in oxidative stress, which ultimately causes endothelial dysfunction, inflammation, and fibrosis in various other tissues [20]. This may result in increased cardiac collagen formation and coronary inflammation, which occur independent of hypertension as suggested by animal studies [21, 22]. In humans, plasma aldosterone levels directly correlate with cardiac collagen content and increases in ventricle wall thickness [23, 24]. This ultimately leads to decreased diastolic function [23, 24]. Additionally there have been increased rates of arrhythmias, myocardial infarctions, strokes, and mortality in patients with PA when compared with matched essential hypertensives [25, 26].

Overactivation of the mineralocorticoid receptor contributes not only to cardiac and cerebrovascular remodeling but also to renal injury [27]. Patients with PA were also found to have higher rates of urinary excretion of albumin than matched essential hypertensives [28, 29]. This was associated with higher creatinine clearance and sonographic evidence of decreased intrarenal vascular resistance. These findings were reversed after adrenalectomy or medical treatment with spironolactone.

In addition to multisystem organ effects, some studies have noted a diminished quality of life in PA [30]. Anxiety and depression that resolve after treatment have also been associated with PA [31]. Undoubtedly, the detection of PA is critical as metabolic derangements, renal, cardiovascular, cerebrovascular effects occur, and early intervention can therefore help prevent these events.

Clinical Presentation

PA can present with symptoms of mineralocorticoid excess or as an incidental adrenal mass in a hypertensive patient. The presence of PA should be suspected in any patient with the triad of hypertension, unexplained hypokalemia, and metabolic alkalosis. The relationship between aldosterone and hypertension has been well studied. Whereas hypertension is defined as a blood pressure greater than 140/90 mmHg, resistant

hypertension occurs when blood pressure remains above goal despite concurrent use of three or more hypertensive agents, one of which is a diuretic [32]. Vasan et al. investigated the relationship between serum aldosterone levels and blood pressure and incidence of hypertension in nonhypertensive patients followed for 4 years [33]. Impressively, per quartile increment in the serum aldosterone level, a 16–17% increase in the incidence of hypertension ($P=0.03$) was observed. Furthermore, the highest serum aldosterone quartile, relative to the lowest, was associated with a 1.61-fold risk of hypertension and a 1.60-fold risk of elevation in blood pressure. Therefore, the authors concluded that increased aldosterone levels albeit within the physiologic range predisposed individuals to the development of hypertension [15, 32–34].

Although the classic presentation of PA is hypokalemia associated with hypertension, hypokalemia is only present in a minority (9–13%) of patients with PA [7, 35, 36]. Symptoms related to hypokalemia include constipation, fatigue, muscle weakness, cramping, and polyuria. Thus, the only symptom required to diagnose hyperaldosteronism is hypertension.

In patients with an adrenal incidentaloma, aldosterone-producing adenoma (APA) accounts for less than 1% of cases [37]. Typically, these nodules are well circumscribed with smooth borders, they are less than 2 cm in diameter, and enhancement is <10 Hounsfield units (HU) on noncontrast CT imaging [37].

Diagnosis

Prompt diagnosis of patients with PA is essential to minimize the morbidity of aldosterone excess. Screening for PA is recommended in patients with the following (Table 8.1): sustained blood pressure measurements greater than 150/100 on each of three measurements obtained on different days, with hypertension (BP > 140/90) resistant to three conventional antihypertensive drugs (including a diuretic), or controlled BP (<140/90) on four or more antihypertensive drugs; hypertension and adrenal incidentaloma; hypertension

and sleep apnea; hypertension and a family history of early onset hypertension or cerebrovascular accident at <40 years of age; hypertension and spontaneous or diuretic-induced hypokalemia; and all hypertensive first-degree relatives with PA [2].

Screening

The ratio of plasma aldosterone concentration (PAC) in ng/dl to plasma renin activity (PRA) ng/ml per hour was first established as a screening test in the early 1980s [38, 39]. Since that time, the widespread use of the aldosterone-to-renin ratio (ARR) as a screening test in patients with normal or low serum potassium and hypertension has led to a 5–15-fold increase in the diagnosis of PA [4].

Although, it's the most reliable screening test for PA, the exact cutoff level for a positive result has varied and many believe that the absolute PAC should also be taken into account [4]. The most commonly used cutoff values that have been used for ARR are 20–30 ng/dl with a sensitivity of 73–93% [40–42]. One of the limitations of the ARR is that it may be elevated in cases of significantly low renin and low-normal plasma aldosterone. Including a minimum PAC minimizes this possibility. In fact, PAC:PRA ratio greater than 20 ng/dl in combination with a

Table 8.1 Indications for the detection and workup of primary aldosteronism

1. Sustained blood pressure measurements >150/100 on each of three measurements obtained on different days, with hypertension (BP > 140/90) resistant to three conventional antihypertensive drugs (including a diuretic) or controlled BP (<140/90) on four or more antihypertensive drugs
2. Hypertension and adrenal incidentaloma
3. Hypertension and sleep apnea
4. Hypertension and family history of early onset hypertension or cerebrovascular accident at <40 years of age
5. Hypertension and spontaneous or diuretic-induced hypokalemia
6. All hypertensive first-degree relatives with primary aldosteronism

Table 8.2 Effect of various antihypertensive medications on plasma aldosterone, renin, and aldosterone-to-renin ratio

Medication	Aldosterone	Renin	ARR
Diuretic	Increased	Increased	Decreased
Angiotensin-receptor blocker	Decreased	Increased	Decreased
ACE inhibitor	Minimal	Minimal	No effect/decreased
β -Blocker	Minimal	Decreased	Increased
α -Blocker	No effect	No effect	No effect
Calcium channel blocker	Minimal	Increased	Decreased
Hydralazine	Minimal	Minimal	No effect
Clonidine	Minimal	Decreased	Increased

plasma aldosterone concentration (PAC) ≥ 15 ng/dl in a morning blood sample obtained when a patient has been out of bed for 2 h has been found to have a sensitivity and specificity of greater than 90% [3, 14, 43]. Importantly, ARR should be repeated if there is a high index of clinical suspicion and a concern that the results may not be accurate given suboptimal screening conditions.

The ARR may be affected by many factors including medications such as mineralocorticoid receptor antagonists and diuretics, posture, time of day, and assay utilized [44, 45]. Antihypertensive medication regimens should be adjusted to avoid interference with testing (Table 8.2). Mineralocorticoid receptor antagonists should not be taken for at least 4–6 weeks prior to testing. Diuretics can often lead to hypokalemia and inhibition of the renin–angiotensin system can be seen with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs). Calcium channel blockers, vasodilators, and alpha-adrenergic blockers have less of an effect on the ARR [46]. Additionally, sensitivity of ARR is improved if blood sample is collected mid-morning from patients that are seated after 2–4 h of upright posture [44]. Renin assays should also be sensitive enough to allow for measurements of 0.2–0.3 ng/ml/h, since ARR is equally dependent on renin as it is for PAC [41]. Thus, appropriate screening utilizing the ARR helps to identify patients with likely PA.

Confirmatory Testing

After a positive screening test, the presence of PA can be confirmed by demonstrating lack of suppression of aldosterone. However, it may not be

needed in the setting of spontaneous hypokalemia, plasma renin below detection levels, as well as PAC >20 ng/dl [2]. Prior to confirmatory testing, patients should avoid any medications that may stimulate renin activity, such as diuretics including potassium-sparing diuretics such as amiloride, eplerenone, and spironolactone [44].

The basic principle of confirmatory testing relies on the fact that patients with hyperaldosteronism are in a state of autonomous hypersecretion of aldosterone, salt retention, and excess natriuresis. Therefore, any additional sodium loading will have no effect on plasma aldosterone. Confirmatory tests include the oral sodium loading test (SLT), intravenous saline suppression test (SST), fludrocortisone suppression test (FST), captopril challenge test (CCT), and furosemide upright test (FUT) [4, 47]. Fludrocortisone acetate, a synthetic mineralocorticoid, 0.1 mg/6 h with NaCl 30 mmol/8 h is administered to the patient [5, 48]. Adequate potassium needs to be administered to ensure that aldosterone secretion is not inhibited by hypokalemia. PA is confirmed if the following criteria are met: PAC measured at 10 am in the upright position is ≥ 6 ng/dl, PRA <1.0 ng/ml/h, normal plasma potassium levels, urinary sodium >3 mmol [5, 48].

Oral salt loading is performed with the administration of a high-sodium diet (300 mmol sodium/day) for 3 days followed by measuring aldosterone and sodium concentrations in the urine. Normal suppression in this case would be a 24-h urinary aldosterone concentration less than 12 μ g/day on the third day [2].

Saline loading test is performed by measuring plasma aldosterone after an infusion of 2 l of 0.9% NaCl over 4 h. A normal response would be a sup-

pressed/low plasma aldosterone concentration (i.e., <5 ng/dl); however, PAC greater than 10 ng/dl is diagnostic for PA. Values in between those mentioned earlier are in the so-called gray zone, although may be considered a positive confirmation for PA by many clinicians [5, 48].

With the captopril challenge test, ARR is measured prior to and after administration of captopril (25–50 mg), which is given after sitting/standing for at least 1 h. Under normal circumstances, captopril, an ACE inhibitor, inhibits the production of aldosterone. However, in PA, aldosterone secretion is autonomous and therefore diagnosis is confirmed when the postcaptopril ARR is more than 30–40 or postcaptopril aldosterone is more than 8.5–15 ng/dl, as plasma aldosterone is normally suppressed by captopril [5].

Recent evidence suggests that N-terminal pro b-type natriuretic peptide (NT-proBNP) could improve the diagnostic evaluation of patients with PA [49]. One study showed that NT-proBNP level positively correlated with the ARR and inversely with the renin level, and is higher in patients with a positive saline load test (confirmation test discussed later) [49]. If validated in additional studies, NT-proBNP may be used as a confirmatory test.

There is no global consensus on a gold standard confirmatory test for PA [2]. Although FST has been considered by some to be the most reliable confirmatory testing, it is expensive, difficult to perform, and requires several days in the hospital [50]. Mularo and colleagues demonstrated that SLT is less expensive and similarly effective as FST for making the diagnosis of PA [51]. SST, when performed in an upright/seated position, may be superior to recumbent SST in terms of sensitivity for detecting PA [52]. When considering SLT, Giacchetti and colleagues observed 100% specificity and positive predictive value for a cutoff value of 7 ng/dl for serum aldosterone at the end of the SLT in patients with an upright ARR of 40 [53]. This same study also noted a slightly higher specificity but lower sensitivity for the postcaptopril ARR. A separate prospective study was conducted to investigate the accuracy of the saline infusion test [54]. Rossi et al, attempted to identify the best aldosterone cutoff

values for differentiating between APA and BAH. However, cutoff values of 6.75 and 6.91 ng/dl, respectively, were only 83% sensitive and 75% specific. There were no true differences in aldosterone cutoffs between BAH and APA. Therefore, although specific for excluding PA saline infusion cannot differentiate between APA and BAH [54].

Captopril challenge has been associated with a high rate of false-positive diagnoses compared to the other tests, and results vary depending on the bioavailability of the drug in different patients [55, 56]. CCT is useful in patients unable to tolerate salt loading/volume expansion, such as those with congestive heart failure or uncontrolled hypertension.

Subtype Classifications

Once the diagnosis is made, the subtype of PA must be established to proceed with optimal treatment. Traditionally, subtypes were classified based on histopathology and included aldosterone-producing adenoma (APA), comprising approximately one-third of cases, bilateral adrenal hyperplasia (BAH), occurring in up to two-thirds of cases (Table 8.3), and aldosterone-producing carcinoma (<1%) [7, 35, 46]. Other less frequent causes include unilateral hyperplasia (1–3%) and familial hyperaldosteronism type I–III (<1%) [46, 57, 58]. Often times, surgical pathology will also reveal an adenoma in the setting of hyperplasia. Unfortunately there is no difference in clinical presentation or outcome based on adrenal histologic findings [59].

Table 8.3 Primary aldosteronism subtypes

Subtypes	Prevalence (%)
Bilateral adrenal hyperplasia (BAH)	65–70
Aldosterone-producing adenoma (APA)	30–35
Unilateral adrenal hyperplasia (UAH)	1–3
Aldosterone-producing adrenal cortical cancer (APACC)	<1
Familial hyperaldosteronism types I–III	<1

Familial Hyperaldosteronism

There are three forms of PA associated with adrenal hyperplasia. These include familial hyperaldosteronism type I (glucocorticoid-remediable aldosteronism, GRA, FH-II), type II (familial occurrence of adenoma or hyperplasia, FH-II), and type III. All three are rare disorders characterized by autosomal dominant inheritance.

Familial Hyperaldosteronism Type I (GRA)

FH-I/GRA is responsible for <1% of PA cases and is inherited in an autosomal dominant fashion with variable penetrance. It is due to a chimeric gene on chromosome 8q of CYP11B1 (11 β -hydroxylase) that is coupled to CYP11B2 (aldosterone synthase gene). The hypersecretion of aldosterone in FH-I depends upon endogenous adrenocorticotrophic hormone (ACTH) secretion, which activates aldosterone synthesis. Although some of the patients present with aldosterone excess, suppressed renin activity, and early onset severe hypertension that is refractory to medical therapy, others have normal blood pressure and are indistinguishable from essential hypertensives [2, 60]. Because the excess aldosterone secretion is activated by ACTH, it can also be suppressed with dexamethasone.

There should be a high index of suspicion in children or young adults who present with a positive family history of severe/resistant hypertension and positive family history of early onset hypertension or hemorrhagic stroke/intracranial aneurysms [61, 62]. Testing for familial hyperaldosteronism type I should be encouraged in younger patients with a family history of hypertension and stroke before 50 years of [7]. Glucocorticoids are the mainstay of treatment.

Familial Hyperaldosteronism Type II

FH-II occurs more frequently than FH-I. The underlying genetics is unclear for FH-II and has also been shown to exhibit genetic heterogeneity [36]. Unlike FH-I, it is nonglucocorticoid reme-

diable [63–65]. FH II is clinically indistinguishable from nonfamilial forms and can present as an adenoma or hyperplasia. Therefore, management is the same as nonfamilial subtypes.

Familial Hyperaldosteronism Type III

FH-III is a new familial form of PA that results in more severe PA, necessitating bilateral adrenalectomy if medical management fails [4]. A germline point mutation in the KCNJ5 gene increases sodium conductance and depolarization of a cell, thereby resulting in constitutive aldosterone secretion [66, 67]. The two main regulators of aldosterone synthesis are serum potassium and angiotensin II, which causes membrane depolarization and influx of calcium through voltage-gated Ca²⁺ channels [66, 67].

Aldosterone-Producing Adenoma (APA)

Aldosterone-producing adenomas occur more often in women than in men [57]. The adenomas are often solitary, unilateral, and <2 cm in transverse diameter (Fig. 8.1) [37]. The majority of APAs are renin unresponsive [68]. Aldosterone-producing adenomas that are renin responsive are composed primarily of zona-glomerulosa-type cells, while those that are renin unresponsive contain zona-fasciculata-type cells [68].

Bilateral and Unilateral Adrenal Hyperplasia

Bilateral adrenal hyperplasia (BAH), also known as idiopathic aldosteronism, is most often micronodular hyperplasia and is the most common subtype of PA. Adrenal glands in these cases may have nodular changes or may even appear normal on CT [2]. Unlike APAs, the majority of BAH cases respond to angiotensin II and therefore should be medically treated. Approximately 1–3% of patients with PA have unilateral adrenal hyperplasia, which can be surgically treated [46].



Fig. 8.1 A surgically resected adrenal gland demonstrating a typical aldosterone producing adenoma (*)

Aldosterone-Producing Adrenocortical Carcinoma (APACC)

Aldosterone-producing adrenocortical carcinoma is the cause of PA in 1% of cases [69]. Patients with aldosterone-producing adrenocortical carcinoma often present with rapid development and progression of symptoms, and commonly there is cosecretion of other steroids. The size of such tumors is variable, and there is no gender or side preference. Metastasis has been reported in 10% of all cases at initial diagnosis and up to nearly half at follow-up and overall prognosis and survival is poor [69].

Subtype Differentiation

Computed Tomography

All patients with PA should have CT imaging performed to evaluate for presence of an adrenal nodule and adrenocortical carcinoma as a cause of aldosterone hypersecretion [2]. The preferred technique for diagnosing an adrenal nodule, is high-resolution CT scanning with 2–3 mm fine cuts, with specificity and sensitivity to detect a nodule approaching 100% in several series [4]. Findings observed on CT imaging in cases of PA include normal appearing adrenal glands, bilateral adenoma, microadenomas (nodules ≤ 1 cm),

or macroadenomas (nodules greater than 1 cm), and minimal unilateral adrenal limb thickening (Fig. 8.2) [41].

In patients with bilateral nodules, CT imaging cannot reliably distinguish between hyperplasia and adenoma [70]. Furthermore, in up to 10% of cases, nonfunctioning adenomas are indistinguishable from true APAs on CT imaging [4]. Furthermore, CT may also be useful in defining the anatomy of the right adrenal vein which is often difficult to cannulate for adrenal venous sampling (AVS) [71].

Adrenal Venous Sampling

Distinguishing between unilateral and bilateral adrenal hypersecretion is paramount in assessing treatment options. Adrenal venous sampling was introduced in the late 1960s as a test to differentiate unilateral from bilateral hyperaldosteronism [72]. Because of technical difficulties in cannulating both adrenal veins and improved imaging modalities such as CT and MRI, it was not a widely accepted practice until multiple studies demonstrated the pitfalls of relying on imaging alone. A recent systematic review evaluated the utility of CT, MRI, and adrenal venous sampling in distinguishing bilateral from unilateral hypersecretion aldosterone in PA [73]. CT/MRI results were discordant with AVS results in 37.8% of

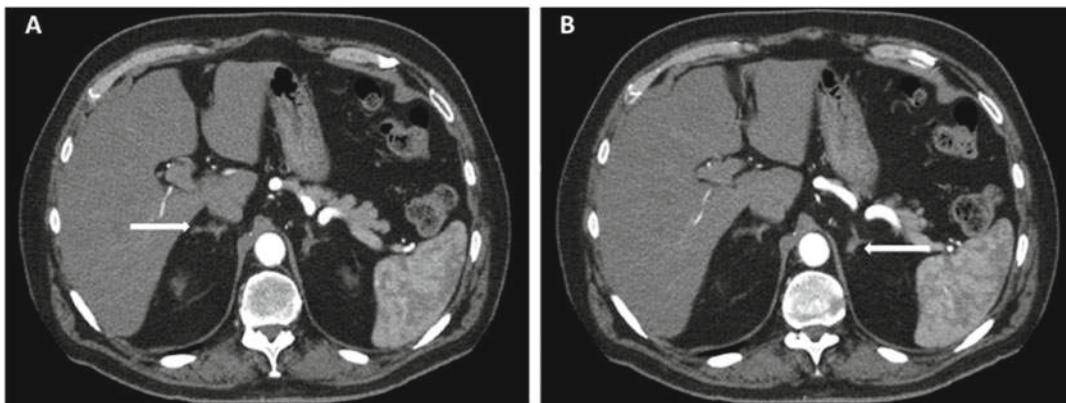


Fig. 8.2 Computed tomography demonstrating multiple right (a) and left (b) microadenomas and a thickened limb on the left adrenal gland

patients. Reliance only on CT/MRI would have resulted in inappropriate exclusion from adrenalectomy in 19.1% of cases where AVS showed unilateral aldosterone secretion, inappropriate adrenalectomy in 14.6% of patients where AVS demonstrated bilateral disease, and adrenalectomy on the wrong side in 3.9% of cases [73]. Thus, inappropriate treatment would have occurred in a significant number of cases if only CT/MRI were used for surgical decision-making. Similarly, a study from the National Institutes of Health demonstrated that patients with a biochemical diagnosis of PA who underwent both CT scan and AVS would have been incorrectly managed in 50% of cases if only CT was used [74]. When evaluating both CT and AVS, a recent study by Salem et al. observed a 73.7% concordant lateralization by CT and AVS in patients with PA [75]. The positive predictive value on CT was less in nonunilateral lesions (50%) compared with unilateral lesions (85%) [75]. Multiple subsequent studies have demonstrated the lower sensitivity and specificity of CT imaging in detecting unilateral adrenal aldosterone excess (78 and 75%, respectively) when compared to that of AVS (95% and 100%, respectively) [71, 76].

AVS is now considered the standard diagnostic modality for differentiating APA from BAH and should be performed by a multidisciplinary team in centers with experience with this modality [46, 72, 77–79]. It should only be performed in patients who screen positive by ARR, are sur-

gical candidates, and wish to undergo surgery. Furthermore, CT scanning followed by AVS is a cost-effective strategy in screening patients with resistant hypertension for PA [80]. Correction of hypokalemia and adjustment of antihypertensive medication regimens should proceed AVS testing. Aldosterone antagonists such as eplerenone or spironolactone should be discontinued for at least 2 weeks prior to testing.

Indications for AVS

Whereas some centers recommend the use of AVS in all patients with PA who wish to undergo surgery, others recommend use of selective criteria such as age >35 [81]. Young and colleagues evaluated surgical cures based on long-term follow-up data on 143 patients who underwent unilateral adrenalectomy for PA. They demonstrated an overall accuracy of CT or MRI for detecting unilateral disease was poor at 58.6%. For patients under the age of 35, the imaging results were 100% concordant with the AVS results; however, there were only five patients under the age of 35 [82]. A recent clinical prediction score study by Küpers et al. sought to identify patients' characteristics that would be useful to predict unilateral aldosterone hypersecretion, thereby allowing patients to proceed directly to surgery without AVS [83]. A cross-sectional study of 101 consecutive patients with PA who underwent AVS was performed at a single institution. AVS was successful in 87 patients and lateralized in 49 patients.

Of the 26 patients with typical Conn's adenoma plus estimated GFR of at least 100 ml/min/1.73 m², or a serum potassium of <3.5 mmol/l, all had unilateral PA. The authors concluded that AVS may potentially be omitted prior to surgery in patients with findings typical of aldosteroma on imaging if they meet two biochemical characteristics (GFR≥100 ml/min/1.73 m² or serum potassium <3.5 mmol/l), with a specificity and sensitivity of 100 and 53 %, respectively [83]. However, larger studies need to be performed to optimally determine selective criteria for who should undergo AVS. Other cases when AVS should not be performed include patients at unacceptable high risk of surgery and those suspected of having adrenocortical carcinoma [81].

AVS Technique

Interventional radiologists catheterize the right and left adrenal veins either sequentially or simultaneously under fluoroscopic guidance by percutaneous access of the femoral vein. Catheterization of the left adrenal vein is typically easily accessed; however, the right adrenal vein is more difficult to catheterize given its short length and acute angle at which it joins the inferior vena cava. Increased expertise with catheterization leads to improved success in right adrenal vein catheterization [2]. The appropriate placement of the catheter tip is verified by contrast venography. Blood samples are then obtained to measure aldosterone and cortisol levels from both adrenal veins and a peripheral vein, which may include the inferior vena cava or an iliac vein. To enhance the sensitivity of AVS, ACTH may be administered either as a bolus (250 µg), continuous infusion (50 µg/h started 30 min before sampling), or a bolus followed by a continuous infusion [81]. Blood samples are then typically collected 5, 10, and/or 15 min after administration.

By using ACTH stimulation, stress-induced fluctuations in aldosterone secretion are avoided and secretion of aldosterone from an aldosteroma is maximized as is the gradient in cortisol from adrenal vein to inferior vena cava to confirm successful cannulation of the adrenal vein [2, 81,

84–86]. Continuous administration of cosyntropin can minimize the pulsatile pattern of secretion of cortisol and aldosterone, which can generate time-related variability in hormone concentrations in blood samples from the adrenal vein [2]. AVS should be performed following an hour of supine rest when performed without cosyntropin stimulation [81]. When cosyntropin is not used, AVS should be performed in the morning hours following recumbency to avoid alterations in aldosterone levels as a result of changes in posture.

Sample Interpretation

In order to correct for dilutional effects of the inferior phrenic vein flowing into the left adrenal vein, an aldosterone-to-cortisol (AC) ratio is calculated for each time point at each site [2]. First the values are used to determine selectivity of each adrenal vein. An adrenal vein-to-peripheral vein cortisol ratio of >2:1 when performed without cosyntropin stimulation and >5:1 when performed with cosyntropin stimulation indicates appropriate placement in each adrenal vein [81].

Once selectivity has been established, the AC ratios are used to determine lateralization. However, there is no clear consensus regarding the best criteria to determine lateralization [87]. Webb and colleagues evaluated ten different criteria identified in the literature to evaluate which one had the best accuracy [87]. They determined that use of ACTH stimulation, confirmation of selectivity with adrenal vein to peripheral vein cortisol ratio >5, and AC ratio of the dominant gland to the nondominant gland of >4 had the best accuracy (Fig. 8.3). In subsequent other studies, the ratio of AC from the hypersecreting gland to the contralateral gland of >4:1 was found to have a sensitivity and specificity of 95 and 100 %, respectively (see Fig. 8.3) [2, 88]. A ratio <3:1 is suggestive of bilateral aldosterone hypersecretion and when between 3:1 and 4:1, either unilateral or bilateral disease is possible. When in the absence of cosyntropin, cortisol-corrected aldosterone ratio of >2:1 is suggestive of unilateral disease [81, 89]. In general, when the aldosterone–cortisol ratio from an adrenal vein is at least 2.5 times greater than that in the peripheral

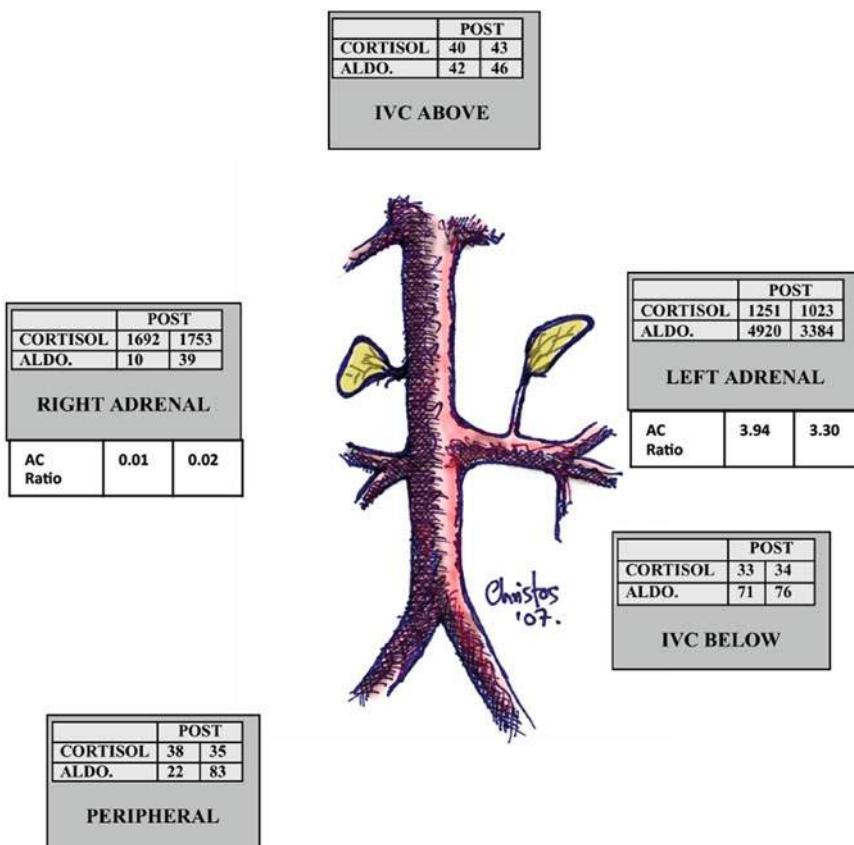


Fig. 8.3 Adrenal vein sampling performed with ACTH stimulation demonstrating aldosterone-to-cortisol ratio of >4:1 from the hypersecreting left adrenal gland. Blood

samples are obtained 5 and 10 min post-ACTH infusion (Image courtesy of Johns Hopkins)

vein, and the aldosterone–cortisol ratio in the contralateral vein is no higher than the peripheral, then AVS has lateralized to a unilateral source of hypersecretion and adrenalectomy is indicated [2].

In PA resulting from BAH, elevated levels of aldosterone production are measured in bilateral adrenal veins in response to stimulation with adrenocorticotropic hormone (ACTH). On the contrary, in the case of an adrenal adenoma, stimulation by ACTH results in a brisk increase in aldosterone in the ipsilateral adrenal vein and reduced levels in the contralateral vein due to negative feedback inhibition [70].

Complications

AVS is both expensive and invasive, making confirmation of a positive PA screening result para-

mount prior to proceeding with this diagnostic modality [7]. Historically complication rates ranged between 5 and 10 %, but more recent studies cite substantially lower rates between 0.2 and 0.9 % [90]. One of the main complications is adrenal vein rupture and hemorrhage, more commonly affecting the right adrenal gland due to difficult vein cannulation as mentioned previously [90]. One multicenter study noted that adrenal hemorrhage was a complication that can occur regardless of years of experience with the technique, with a median duration of experience of 5 years [90]. When it occurs, adrenal hemorrhage usually causes either no or inconsequential effects on adrenal function. Furthermore, none of the cases reported in this study required intervention to control the bleeding [90]. Adrenal infarction, thrombosis, hypertensive crisis, and adrenal

insufficiency have also been reported [71]. These complications are rarely associated with long-term sequelae, although they may make laparoscopic surgery more difficult [71]. If AVS is unsuccessful, it may be repeated. Alternatively, posture stimulation testing is sometimes used in patients when a CT scan demonstrates a unilateral adrenal mass but AVS is unsuccessful in localizing [2]. This is based on the premise that BAH is affected by changes in angiotensin II that results with standing, whereas the PAC in patients with an aldosteroma shows diurnal variation and is unaffected by changes in angiotensin II [91].

Other Diagnostic Localizing Modalities

Iodocholesterol scintigraphy, although still used in some countries, is no longer used in the United States. Since its sensitivity depends on the size of an adenoma, it is less useful for small adenomas that would result in poor tracer uptake [92, 93]. Additionally, recent studies have shown that 18-hydrocycortisol, 18-oxocortisol, and 18-hydroxycortisone (formed by 18-hydroxylation of corticosterone) are elevated in patients with unilateral aldosterone-producing adenoma, and that 18-oxocortisol measurements may be used to distinguish between APA and BAH [94, 95]. However, such measurements have not been validated to guide surgical management, and larger studies are needed to determine its role to supplement AVS [2].

Genetic Testing

Patients with a positive family history of PA, stroke at a young age (<40), or an onset of confirmed PA at less than 20 years of age should undergo genetic testing for hyperaldosteronism FH-I [96, 97]. Southern blotting and polymerase chain reaction (PCR)-based methods may be used to detect the hybrid gene in FH-I [98, 99]. PCR is much quicker and avoids the use of radioactive isotopes, and therefore is the preferred

genetic test for most. In very young patients with PA, KCNJ5 germline mutation testing should be performed to evaluate for FH-III. In contrast to FH-I and FH-III, the molecular basis of FH-II is unclear and more complex to warrant genetic evaluation for clinical decision-making.

Causative somatic mutations in several key proteins of adrenal zona glomerulosa cells have recently been identified in aldosterone-producing adenomas. These include mutations in KCNJ5 gene (encodes the G-protein-activated inward rectifier K⁺ channel 4, GIRK4), CACNA1D (encodes a voltage-gated calcium channel), ATP1A1 (encodes for the alpha subunit of the Na⁺/K⁺-ATPase), and ATP2B3 (encodes for the plasma membrane calcium-transporting ATPase 3) [67, 100–107]. More recently, Teo and colleagues described a possible association between CTNNB1, a gene encoding β-catenin in the Wnt cell-differentiation pathway, and pregnant patients with aldosterone-producing adenomas [108]. However, other investigations that followed also describe CTNNB1 mutations in nonpregnant women with aldosterone-producing adenomas [109].

Treatment

Surgery

For patients with a confirmed diagnosis of PA from unilateral hypersecretion, a minimally invasive adrenalectomy is recommended (Fig. 8.4) [2]. Surgical resection has been shown to be more cost effective than medical management for PA due to APA [110, 111]. When compared to open surgery, minimally invasive resection avoids a large painful incision, is associated with less blood loss, decreased analgesic requirements in the early postoperative period, faster recovery, and decreased length of stay [112–117]. Furthermore, minimally invasive approaches are more cosmetically appealing to patients.

Minimally invasive approaches include laparoscopic or robotic transabdominal, or retroperitoneoscopic adrenalectomy [118–120]. In contrast to transabdominal approaches, retroperitoneoscopic

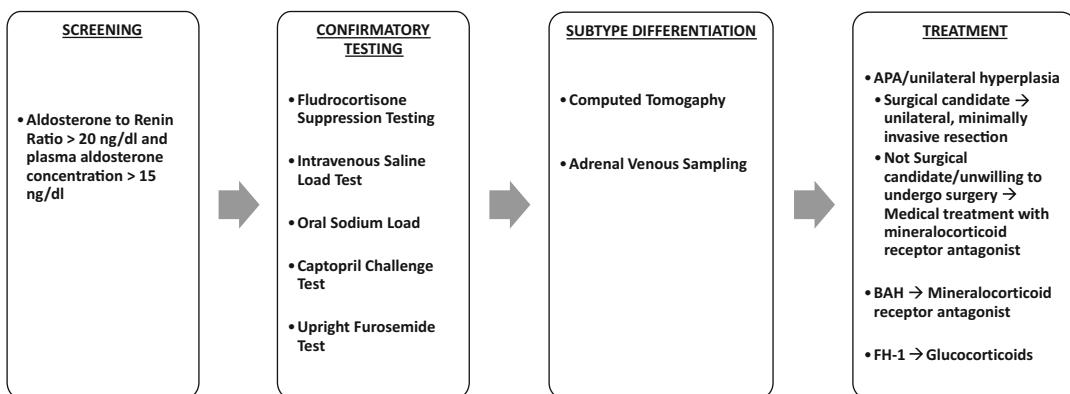


Fig. 8.4 Workup for primary aldosteronism

adrenalectomy avoids the need to mobilize intraperitoneal organs by using an extraperitoneal posterior endoscopic approach that provides direct access to the gland and may be useful in cases of bilateral adrenal pathology [121]. Also, it is advantageous in patients with multiple prior abdominal surgeries, as this approach therefore reduces complications associated with bowel injury and postoperative adhesions [118]. Disadvantages of such an approach include a less familiar posterior view of the anatomy, increased risk of complications related to increased insufflation in a small working space such as pneumomediastinum/pneumothorax, and inadvertent intrathoracic port placement.

Robotic surgery is gaining popularity in the approach for adrenal disease, given the improved surgeon ergonomics, 3D visualization, and magnification of the anatomic structures. However, a learning curve is associated with the technique. Furthermore, many surgeons would argue that the traditional laparoscopic approach produces similar outcomes as robotic resection, without the increased costs associated with the complex setup of robotic surgery. Ultimately, the preferred approach should be guided by the surgeon's familiarity and experience with the technique.

Adrenalectomy for unilateral hypersecretion cures hyperaldosteronism, improves associated hypertension, corrects hypokalemia, and improves glucose homeostasis. Surgical cure of hypertension has been associated with several patient-related factors: shorter duration of hyper-

tension (<5–10 years), ≤2 antihypertensive medications, higher preoperative blood pressure, preoperative normal renal function, higher preoperative plasma ARR, younger patients, female sex, body mass index (BMI) ≤25 kg/m², lack of family history of hypertension, and no evidence of vascular remodeling [2, 122–124]. In fact, an Aldosteronoma Resolution Score (ARS) was developed by Zarnegar et al. to predict likelihood of cure following resection; this was based on BMI <25 kg/m², female sex, duration of hypertension ≤6 years, and number of preoperative antihypertensive medications ≤2 [125]. Points were assigned to each of the aforementioned variables, and a composite score ≥4 predicted a high likelihood of resolution of hypertension. When they applied the ARS to 47 patients with a median follow-up of 1135 days, 73 % of those with an ARS 4–5 had complete resolution of their hypertension [126]. Although originally used to predict response within a year, additional investigation has demonstrated that the ARS may predict the probability of resolution of hypertension further out, beyond a year [126].

When compared to medical therapy in BAH, surgery in unilateral APA has been reported to be more beneficial with regards to cardiovascular outcomes and quality of life [127–130]. Catena and colleagues observed significantly decreased left ventricular mass in a prospective follow-up study of 1 year only in patients undergoing adrenalectomy [130]. Other studies have demonstrated improvement in hypertension and reduced

all-cause mortality in patients with unilateral PA treated with adrenalectomy compared with medical treatment [131, 132]. Using the SF-36 well-validated quality of life questionnaire, Sukor et al. observed significant improvement at 3 months following laparoscopic adrenalectomy with regards to physical functioning, physical and emotional well-being, general health, mental health, and vitality [30].

Postoperative Management

Following surgical resection, biochemical response should be evaluated by measuring baseline plasma renin activity and aldosterone levels. Antihypertensive therapy should be gradually weaned postoperatively, as the patient will have improved blood pressure without the stimulus for aldosterone hypersecretion and may not require any long-term pharmacologic control. Generally, blood pressure will improve anywhere from 1 to 6 months, and decreases in blood pressure can be noticed up to a year following surgery [3, 106]. Duration of preoperative hypertension will also determine the ability to completely wean off antihypertensive medications successfully.

In general, on the first day following surgery, potassium supplementation and spironolactone should be discontinued [3]. Also, unless potassium levels are slow to return to normal postoperatively, intravenous fluid should not contain potassium. Refractory hyperkalemia has been reported following adrenalectomy in patients with aldosterone-producing adenoma [133–135]. Hyperkalemia typically develops due to contralateral zona glomerulosa insufficiency and inability of the contralateral adrenal gland to secrete sufficient amounts of aldosterone. Risk factors associated with postoperative hyperkalemia after adrenalectomy for APA include longer duration of hypertension (≥ 9.5 years), larger mass size on pathology (≥ 1.95 cm), impaired preoperative renal function (GFR < 58.2 ml/min), and older age (≥ 53 years) [133]. Furthermore, adrenalectomy, in a small percentage of cases, may result in persistent hypoaldosteronism that requires mineralocorticoid replacement therapy with fludrocortisone [135].

Medical Treatment

Treatment with mineralocorticoid antagonists is recommended in patients BAH or those with APA who are unwilling/unable to undergo surgery (see Fig. 8.4). It is the preferred treatment in BAH, as surgery is associated with only a 19% cure rate [5]. Medical therapy has been shown to improve endothelial function, reduce blood pressure in resistant hypertension, decrease albuminuria in patients with diabetes, and reduce mortality in patients with cardiovascular disease [136, 137]. Mineralocorticoid antagonism with spironolactone (nonselective antagonist) or eplerenone (selective) is recommended for patients with PA resulting from bilateral adrenal hyperplasia [138]. Eplerenone is a newer, selective mineralocorticoid antagonist that has a higher cost, but better tolerability profile than spironolactone. Its potency is half that of spironolactone, and its administration is twice daily given its shorter half-life. Changes from baseline in diastolic blood pressure may be less in patients treated with eplerenone compared with spironolactone [138].

Amiloride and triamterene are two available sodium channel antagonists that prevent the downstream effects of aldosterone and may be used as a treatment for PA. Overall, although less efficacious than spironolactone, each may have its own advantages. Amiloride is a potassium sparing diuretic, and therefore helps correct the potassium imbalance present and also lacks the adverse endocrine side effects [139]. Other antihypertensives that may be used in addition to spironolactone or other mineralocorticoid antagonists to aid blood pressure control efforts include calcium channel blockers, vasodilators, ACE inhibitors, and angiotensin receptor blockers.

Ghose et al. evaluated patients with documented aldosterone-producing adenomas who were treated medically with spironolactone or amiloride for at least 5 years [140]. From the period of diagnosis up until last follow-up, diastolic blood pressure decreased from 106 to 79 mmHg and systolic blood pressure from 175 to 129 mmHg, and serum potassium concentration

increased from 3.0 to 4.3 mmol/l [140]. Side effects of spironolactone therapy are dose dependent and include muscle cramps, gynecomastia, and erectile dysfunction. A double-blind randomized study demonstrated a higher incidence of male gynecomastia and female mastodynia in patients randomized to spironolactone versus eplerenone [138]. Eplerenone is better tolerated as it does not have the progesterone agonist and antiandrogen effects [141]. Amiloride or triamterene may be used in addition to spironolactone, in order to avoid the need for higher doses and associated side effects.

FH-I/GRA should be treated with the lowest dose of glucocorticoid at bedtime, preferably longer acting ones as dexamethasone or prednisone, to suppress the early morning ACTH surge. Usually, dexamethasone not exceeding 0.5–0.75 mg/day is sufficient to obtain good blood pressure control [48]. At times, a mineralocorticoid needs to be added to the regimen to facilitate further lowering of blood pressure to goal. The administering provider should make sure that the doses are tolerable and avoid overtreatment that would result in steroid excess side effects (e.g., Cushing's and growth retardation).

Conclusions

PA is regarded as a major public health issue, with significant associated cardiovascular, cerebrovascular, renal, and end-organ morbidity. However, it is the most frequent curable form of secondary hypertension. PA most commonly results from an oversecretion of aldosterone from bilateral adrenal hyperplasia or an adrenal adenoma, but can also arise from unilateral hyperplasia, adrenocortical carcinoma, and familial hyperaldosteronism subtypes. With the increasing use of ARR over the past several decades, the incidence of PA has been on the rise and clinicians are able to diagnose and intervene early. Adrenal vein sampling should be performed to distinguish unilateral from bilateral disease in any patient who is a surgical candidate. Whereas surgery is the mainstay of treatment for unilateral aldosterone excess, medical management with

mineralocorticoid receptor antagonism is recommended for bilateral adrenal hyperplasia and in cases when surgery is not pursued. In the majority of cases, surgery for unilateral PA results in biochemical cure, with significant improvements in blood pressure control and quality of life in other cases. Similarly, medical management reduces the risk of cardiovascular, renal, and other end-organ damage. Despite the many improvements made in the workup and management of PA, more can be done to help provide a uniform approach to the disease to help further facilitate early diagnosis and intervention.

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Cushing Syndrome: Presentation, Diagnosis, and Treatment, Including Subclinical Cushing Syndrome

Vladimir Neychev

Presentation

Clinical Picture

The ultimate physiological role of the hypothalamic–pituitary–adrenal (HPA) axis is to regulate the synthesis and secretion of cortisol by the adrenal cortex [1]. This highly controlled, circadian process involves secretion of corticotrophin-releasing hormone (CRH) by the hypothalamus that causes release of pituitary adrenocorticotrophic hormone (ACTH), which finally stimulates the adrenal gland to produce cortisol. Cortisol in turn exerts control on the HPA axis by a negative feedback mechanism and suppression of the activity of hypothalamus and pituitary glands (Fig. 9.1).

Dysfunction or dysregulation of HPA axis at any level can lead to cortisol overproduction and, eventually, to endogenous hypercortisolism. Chronic exposure of the body to excess cortisol defines the clinical phenotype of Cushing syndrome (CS) with signs and symptoms that can be

grouped into three major categories: (1) physical features such as central obesity, muscle wasting and weakness, acne, hirsutism, and wide (>1 cm) purple striae; (2) metabolic comorbidities including arterial hypertension, diabetes, menstrual irregularities, libido abnormalities, and osteoporosis; (3) neurobehavioral abnormalities such as anxiety, depression, mood change, and memory loss [2, 3].

Although the occurrence of any single feature ranges very widely among published series, certain clinical features such as central obesity with rapid enlargement of the trunk, abdomen, and face; large purple striae from excessive skin stretch and thinning; proximal muscle wasting with associated weakness and fatigue; and easy bruising prevail in adult patients with CS (Table 9.1) [4–6]. The clinical manifestation of the syndrome in children and adolescent is different from that in adults (see Table 9.1). Development of generalized obesity (in contrast to central obesity usually seen in adults), linear growth retardation, fatigue, and precocious puberty are among the dominant clinical features in children [8, 14, 26–29].

However, the manifestation of this heterogeneous and complex disorder is, in part, related to the extent and duration of hypercortisolism and, thus, the clinical spectrum can range broadly from subclinical or mild variants to severe florid forms [4, 18, 30]. Patients with severe hypercortisolism usually present with a combination of a number of signs and symptoms often from all three major

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Fig. 9.1 Schematic of the functional organization of hypothalamic–pituitary–adrenal axis. CRH corticotrophin-releasing hormone, ACTH adrenocorticotrophic hormone

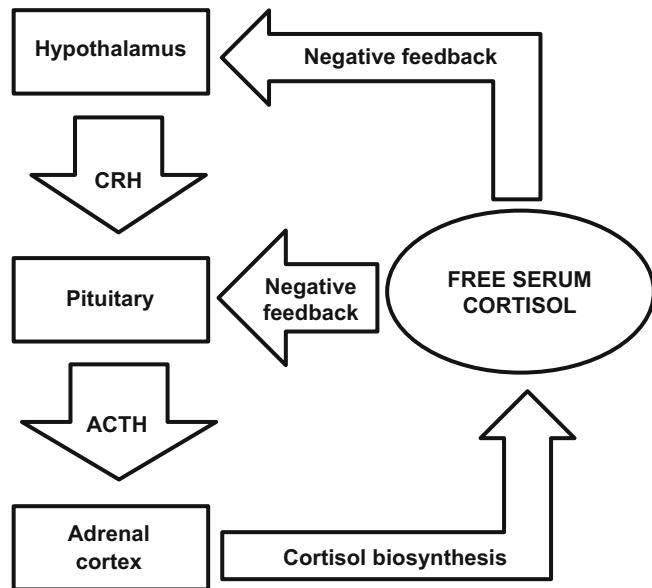


Table 9.1 Clinical presentation of Cushing syndrome

Initial presenting signs and symptoms	Proportion		References
	Adults	Children and adolescent	
Obesity/weight gain	Central	Generalized	Feeelders et al. [7]
	90–96 %	90–100 %	Joshi et al. [8]
Thin skin with large purple striae	62–85 %	53 %	Bascaro et al. [5] Storr et al. [9]
Growth retardation		43–96 %	Joshi et al. [8] Savage and Storr [10]
Hypertension	70–85 %	32–50 %	Isidori et al. [11] Lodish et al. [12]
Proximal muscle wasting/weakness	60–82 %	64 %	Sharma et al. [13] Chan et al. [14]
Menstrual irregularity in women	70–82 %		Lado-Abeal et al. [15] Kaltsas et al. [16]
Decreased libido	25–80 %		Nieman and Ilias [2] Valassi et al. [17]
Hirsutism/virilization	70–75 %	48–60 %	Chan et al. [14] Newell-Price et al. [18]
Precautious puberty		44–53 %	Dupuis et al. [19] Stratakis [20]
Glucose intolerance/diabetes	32–80 %	13 %	Arnaldi et al. [21] Lonser et al. [22]
Osteoporosis/fractures	30–50 %	7 %	Sharma et al. [13] Lonser et al. [22]
Delayed bone age		88 %	Peters et al. [23]
Neuropsychiatric symptoms	54–79 %	19–51 %	Tang et al. [24] Sonino et al. [25]

categories and this so-called classical clinical CS phenotype is practically unmistakable [6]. On the other hand, the clinical picture of mild or cyclic variants may not be as revealing and patients may present with fewer, sometimes, isolated signs and symptoms.

In subclinical CS associated with functionally active adrenal incidentalomas, patients have biochemical evidence of excess cortisol without overt clinical signs or symptoms of CS and will appear clinically normal [31–36]. In cyclic CS, patients may present with a pattern of episodic secretion of excess cortisol with peaks occurring at intervals of several days to many months and also may have intermittent or constant clinical features [37]. The features that are common in patients with CS such as obesity, hypertension, glucose intolerance, osteoporosis, depression, and anxiety are also common in the general population or in patients suspected to have CS in whom it was excluded. This latter condition is also known as pseudo-Cushing syndrome (pseudo-CS), because patients may have clinical features compatible with CS, and sometimes even biochemical signs, but do not have endogenous pathologic hypercortisolism [6].

Thus, establishing the diagnosis especially in milder or subclinical cases may not always be straightforward and patients may remain undiagnosed or misdiagnosed for a long period of time [21, 33, 38]. In such cases, an important clinical clue to suspect glucocorticoid excess is that CS tends to progress with development and accumulation of relevant features with increasing severity over time [18, 34].

Epidemiology and Etiology

CS is a rare disorder with a female-to-male ratio of 3:1, an estimated annual incidence of 2–5 cases per million people, and a prevalence of 39–79 cases per million; approximately 10% of these occur in children [17, 20, 28, 30, 39, 40]. According to the underlying pathophysiological mechanisms, CS can generally be subdivided into ACTH-dependent (~80%) and ACTH-independent (~20%) causes (Table 9.2). Approximately 80–85% of ACTH-

dependent CS cases are caused by ACTH secreting pituitary adenomas, a condition also known as Cushing disease (CD) [18, 41, 42]. These ACTH secreting tumors are almost always benign and are usually microadenomas (<1 cm in diameter). The remaining 15–20% of ACTH-dependent CS are due to extra-pituitary paraneoplastic ACTH secretion (ectopic ACTH syndrome) by tumors such as small-cell carcinoma of the lung, bronchial carcinoid, pheochromocytoma, pancreatic neuroendocrine tumors, and medullary thyroid carcinoma [43, 44]. In very rare occasions, excess pituitary ACTH production is caused by tumors secreting CRH.

Unilateral functional adrenocortical adenomas account for the majority (~80%) of ACTH-independent CS cases, while adrenocortical carcinoma is the underlying cause in approximately 15% of cases [13, 21]. An important feature of adrenocortical carcinoma is its bimodal age distribution, with peaks in childhood and in the fourth to fifth decades of life. While the majority of cases of this devastating disease are sporadic, one should bear in mind that this cancer can be part of hereditary syndromes such as the Li–Fraumeni syndrome, Beckwith–Wiedemann syndrome, multiple endocrine neoplasia type 1, congenital adrenal hyperplasia, familial polyposis coli, β -catenin mutations, and germline TP53

Table 9.2 Etiology of Cushing syndrome

Pathophysiological cause of CS	Proportion
ACTH dependent	70–80 %
Cushing disease	60–70 %
Ectopic ACTH	5–10 %
Ectopic CRH	Rare
ACTH independent	20–30 %
Unilateral adrenal disease	15–29 %
Adrenocortical adenoma	10–22 %
Adrenocortical carcinoma	5–7 %
Bilateral adrenal disease	1–2 %
Macronodular adrenal hyperplasia	<2 %
Micronodular adrenal hyperplasia / PPNAD	<2 %

CS Cushing syndrome, ACTH adrenocorticotrophic hormone, CRH corticotrophin-releasing hormone, PPNAD primary pigmented nodular adrenocortical disease

mutations without Li–Fraumeni that is frequent in southern Brazilian children [45, 46].

Among the less common causes of ACTH-independent CS are the familial and sporadic forms of bilateral macronodular (BAIMAH) and micronodular adrenal hyperplasia with its pigmented variant also referred to as primary pigmented nodular adrenocortical disease (PPNAD) [3, 18, 30].

In children before the age of 7 years, the primary adrenal causes of CS including adenoma, carcinoma, BAIMAH, and PPNAD are most common, whereas CD accounts for approximately 75 % of all pediatric CS cases after that age [20]. Unilateral adrenal tumors presenting with CS in young children (under age of 5 years) are usually malignant (more than 70 % of cases) [20]. Ectopic ACTH secreting tumors are extremely rare in children and can be seen in less than 1 % of pediatric CS cases [20, 30]. In children with a known family history of hereditary cancer syndromes such as Carney complex, multiple endocrine neoplasia type 1, hereditary leiomyomatosis/renal cancer syndrome, and McCune–Albright syndrome, endogenous hypercortisolism and CS can be the first sign and manifestation of the syndromes [47, 48].

Diagnosis

In general, the diagnostic workup of CS involves three steps: (1) careful history and a thorough physical examination looking for the characteristic signs and symptoms of CS (see Section “Clinical Picture”) while excluding exogenous CS causes; (2) laboratory investigations to document the presence of hypercortisolism (the episodic or cyclic nature of some CS cases must be taken into consideration when utilizing biochemical screening tests) (see Sections “Initial Screening: First-Line Biochemical Tests” and “Additional Tests: Second-Line Tests”), and; (3) determining the underlying pathophysiological cause that is essential for outlining the management strategy (see Sections “Differential Diagnosis of CS Pathophysiological Causes” and “Management”) [5, 21].

The current Endocrine Society’s Clinical Practice Guideline for the diagnosis of Cushing syndrome recommends that testing should be performed in patients with multiple and progressive features highly suggestive of the syndrome (see Section “Clinical Picture”), patients with adrenocortical incidentalomas, patients with unusual features for their age (e.g., hypertension, osteoporosis), and children with decreasing height percentile and increasing weight [4].

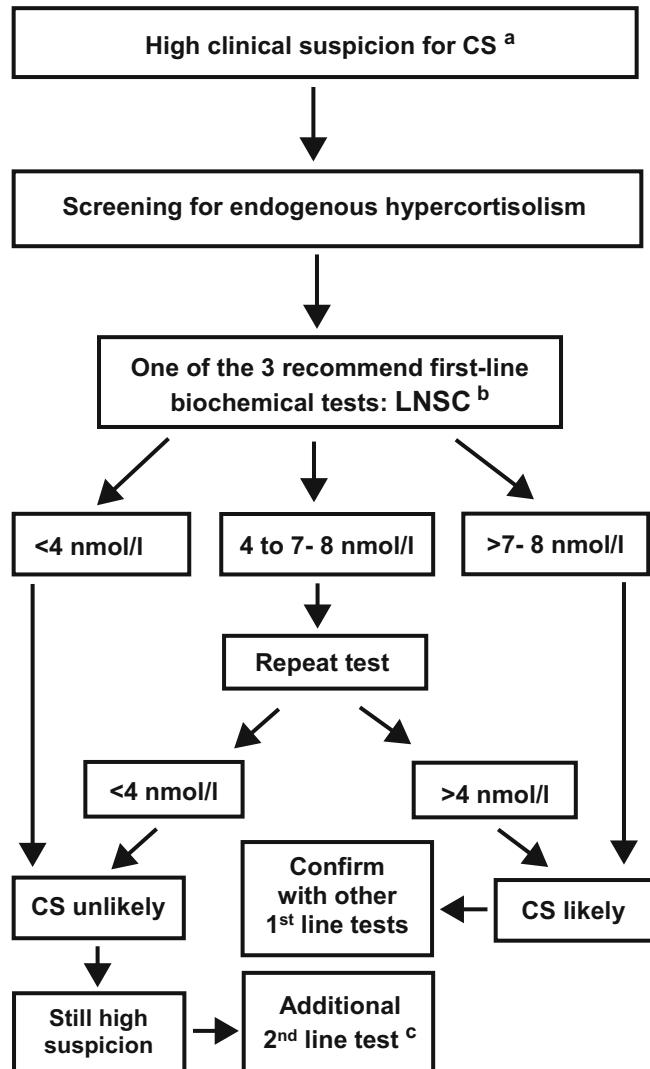
Initial Screening: First-Line Biochemical Tests

The three general pathophysiological principles that are utilized in biochemical diagnosis of hypercortisolism are failure to attain the normal nadir in circadian rhythm of cortisol secretion, loss of sensitivity of ACTH-secreting tumors to cortisol negative feedback, and increased cortisol biosynthesis with the resultant abnormal excretion of free cortisol in the urine [1]. Accordingly, the three first-line biochemical screening tests recommended for the diagnosis of endogenous hypercortisolism are used to specifically interrogate these pathophysiological aspects: (1) measurement of late-night salivary cortisol (LNSC) is used to evaluate circadian rhythm and the presence or absence of normal nadir of cortisol secretion after bedtime, (2) low-dose dexamethasone suppression (LDDS) test is used to assess attenuated sensitivity to glucocorticoid negative feedback, and (3) 24-h urine free cortisol (UFC) that evaluates for the overproduction of cortisol (Fig. 9.2) [1, 4].

Late-Night Salivary Cortisol

In healthy individuals, the level of serum cortisol reaches a peak in the morning (between 0700 and 0900 h) and then falls to achieve a nadir between bedtime and 0200 h, while in patients with CS this diurnal phenomenon is lost [4, 6]. The failure to achieve nadir in cortisol secretion is one of the earliest biochemical signs of CS of any etiology [49]. Although it has been shown that detecting increased midnight serum cortisol level has high sensitivity for patients with CS, obtaining a

Fig. 9.2 Screening algorithm for the diagnosis of endogenous hypercortisolism and Cushing syndrome. ^a Patients with multiple and progressive features highly suggestive of Cushing syndrome (CS) (see Section “Clinical Picture”), patients with adrenocortical incidentalomas, patients with unusual features for their age (e.g., hypertension, osteoporosis), and children with decreasing height percentile and increasing weight. ^b The three first-line biochemical screening tests recommended for the diagnosis of endogenous hypercortisolism are: (1) Measurement of late-night salivary cortisol (LNSC). The test should be performed twice. (2) Low-dose dexamethasone suppression (LDDS) test. (3) 24-h urinary free cortisol (UFC). The test should be performed at least twice. ^c Combined 48 h LDDS test followed by stimulation with corticotrophin-releasing hormone



stress-free late-night blood sample in routine outpatient clinical practice is not always feasible [1, 38].

On the other hand, the concentration of serum-free cortisol is in equilibrium with the concentration of salivary cortisol that is not affected by the rate of saliva production [50, 51]. In addition, saliva is easy to collect and cortisol is stable at room temperature that allows the samples to be mailed to the medical office or laboratory for analysis [6]. These advantages make the LNSC a suitable, convenient, and reliable procedure for both inpatient and outpatient screening for CS

with a 90–100% sensitivity and a 92–100% specificity [52–55].

The salivary samples for the LNSC test must be collected on two separate evenings between 2300 and 2400 h either by using a cotton pledge in the mouth and chewing for 1–2 min or by passive drooling into a plastic tube [6]. Normal subjects usually have salivary cortisol levels of less than 145 ng/dl (4 nmol/l) between 2300 and 2400 h [4]. Salivary cortisol levels consistently greater than 7.0 nmol/l make the diagnosis of CS very likely, while values between 4.0 and 7.0 nmol/l prompt additional biochemical confirmation [56].

Although the precise mechanisms underlying the effects of coexisting medical conditions, age, and lifestyle on salivary cortisol concentrations have not been completely understood, it is important to note that LNSC test may give false-positive results in elderly patients, shift workers, or people crossing widely different time zones with hampered circadian rhythm, and in critically ill patients [4, 6, 57].

Low-Dose Dexamethasone Suppression

In contrast to normal subjects in whom the administration of a supraphysiological dose of glucocorticoid results in suppression of secretion of ACTH and cortisol, there is a failure of this suppression in patients with endogenous CS when the synthetic glucocorticoid dexamethasone is given [58].

The overnight and the 48 h dexamethasone-suppression tests are the two most widely used LDDS tests: 1 mg of dexamethasone is given at 2300 h in the overnight test and the serum cortisol is measured the next day at 0800–0900 h; in the 48 h test, 0.5 mg of dexamethasone is given every 6 h for 2 days at 0900, 1500, 2100, and 0300 h with measurements of serum cortisol at 0900 h at the start and end of the test [18]. To achieve sensitivity rates for CS of greater than 95%, experts in the field have recommended a cutoff for suppression of the serum cortisol after dexamethasone administration to less than 1.8 µg/dl (50 nmol/l) [4, 21, 59].

For pediatric patients weighing more than 40 kg, the initial adult protocol described earlier and the adult threshold for normal suppression are used, while for children with body weight less than 40 kg, the dose is adjusted to 30 µg/kg per day and given in divided doses [26]. Consensus statement of the experts in the field suggests using LDDS or LNSC tests, rather than UFC in patients suspected of having mild CS [4].

It has to be noted, however, that 3–8% of patients with CD will display normal sensitivity [less than 50 nmol/l] with false-negative results on either LDDS test, while false-positive results can be seen in up to 30% of tests performed in patients with other medical conditions or in

healthy individuals [18, 60]. Hence, repeated tests and/or other investigations may be required if clinical suspicion of CS remains high [18]. In addition, caution needs to be used when performing these tests in patients with potential gastrointestinal malabsorption or those taking medications that increase hepatic clearance of dexamethasone (e.g., carbamazepine, phenytoin, phenobarbital, or rifampicin) or increase synthesis of cortisol-binding globulin (e.g., estrogens) [54]. Patients with liver or renal failure may have reduced dexamethasone clearance that may also interfere with LDDS test results.

Twenty-Four Hour Urine Free Cortisol

Compared to the two methods described earlier (see Sections “Late-night Salivary Cortisol” and “Low-Dose Dexamethasone Suppression”) that evaluate total serum cortisol level, UFC provides a unique perspective on pathophysiology of cortisol metabolism in that it measures the excess circulating cortisol that is filtered and excreted in the urine as free cortisol. Another advantage is that UFC provides an integrated assessment of cortisol secretion over a 24-h period [4]. It must be noted, however, that the type of assay technique used for UFC measurement may affect whether a patient with mild CS will have a normal or abnormal UFC [6, 61, 62]. For instance, the cross-reactivity with cortisol precursors and metabolites that is seen in antibody-based immunoassays such as ELISA immunoassay is not seen in the structurally based assays such as high performance liquid chromatography or tandem mass spectrometry [49]. Thus, patients may have an abnormal result in the immunoassay, but a normal result in the structurally based assay [49, 63].

The experts’ recommendation is to use the upper limit of normal range for the particular UFC assay as the cutoff criterion for a positive test in order to achieve high sensitivity, and to perform the test twice, at least once (adult normal ranges may be used for pediatric patients) [4]. However, the interpretation of the results could be challenging due to the above-mentioned differences in assay methodology, and, while specificity of UFC for CS may approach 100%, the sensitivity may be as low as 45–71% [38].

There are some important caveats to the test including its inconvenience and the possibility of urine under- or overcollection. Because high fluid intake (more than 5 l/day) can falsely increase and creatinine clearance less than 60 ml/min can falsely decrease UFC results, patients must be well instructed and able to comply with the correct collection procedures [64, 65]. In addition, the measurement of creatinine and urine volume need to be performed in order to assess completeness with more than two UFC measurement often required to avoid false results, detect cyclic hypercortisolism, and validate the diagnosis [66]. Use of UFC or LNSC tests rather than LDDS is suggested by the experts' consensus statement in patients suspected of having cyclic CS or in the initial evaluation of pregnant women suspected of having hypercortisolism [4].

A complete 24-h urine collection with appropriate total volume and urinary creatinine levels requires patient and/or patient's family education using clear instructions. The procedure begins after the first morning void is discarded with all subsequent voids throughout the day and night included and urine collection kept refrigerated. It has to be noted that the sample is considered complete only when the first morning void on the second day has been collected in the urine sample container.

Additional Tests: Second-Line Tests

The current clinical practice guideline for the diagnosis of CS recommends that in cases with high pretest probability of CS but normal first-line test results, use of an additional alternative tests (second-line tests) has the potential benefit of differentiating patients with mild CS from normal individuals and those with pseudo-CS [4].

Dexamethasone Suppression Test Combined with CRH Stimulation

The combined 48 h LDDS test followed by CRH stimulation (LDDS-CRH) is performed by giving 0.5 mg dexamethasone orally every 6 h for 48 h,

starting at 1200 h, and then (2 h after the last dose of dexamethasone) administering 1 µg/kg CRH intravenously at 0800 h. The plasma cortisol value 15 min after CRH is usually greater than 1.4 µg/dl (38 nmol/l) in patients with CS, but remains suppressed in normal individuals and in patients with pseudo-CS [5, 13, 21].

Midnight Serum Cortisol

In patients with a high clinical index of suspicion of CS who had normal or equivocal first-line screening results a sleeping midnight serum cortisol of greater than 1.8 µg/dl increases the probability of CS. It has to be noted that children may have their cortisol nadir earlier than midnight [67]. In order to avoid false-positive responses due to the stress of hospitalization, patient needs to be admitted to the hospital for a period of at least 48 h prior to the test. The blood sample must be drawn within 5–10 min of waking the patient, or through an indwelling line, to avoid false-positive results [68]. In order to obtain a night sleep sample for serum cortisol in children, placement of an IV line for blood drawing before bedtime is essential [4].

Differential Diagnosis of CS Pathophysiological Causes

After the diagnosis of hypercortisolism has been established by the biochemical test described earlier (see Sections "Initial Screening: First-Line Biochemical Tests" and "Additional Tests: Second-Line Tests"), the next step is to establish the pathophysiological cause of CS (Table 9.3).

Assessment of ACTH

Measurement of plasma ACTH concentration at 0900 h represents the first step and the best way to distinguish ACTH-dependent from ACTH-independent CS (see Table 9.3). To avoid falsely low results, the ACTH assay requires collection of blood into a precooled EDTA tube, placement on ice, and rapid delivery to the laboratory for refrigerated centrifugation, because ACTH is degraded quickly by plasma proteases [21].

Table 9.3 Results of diagnostic tests to identify cause of Cushing syndrome

Test	Pathology		
	Adrenal	Pituitary	Ectopic
Plasma ACTH	Low	High or normal	High
CRH stimulation	No response	Response	No response
HDDS	No suppression	Suppression	No suppression
Pituitary CT/MRI	Normal	Tumor	Normal
Adrenal CT/MRI	Unilateral or bilateral tumor	Normal/bilateral hyperplasia	Normal/bilateral hyperplasia
BIPSS	Not indicated	Central-to-peripheral gradient	No central-to-peripheral gradient

ACTH adrenocorticotrophic hormone, CRH corticotrophin-releasing hormone, HDDS high-dose dexamethasone suppression, CT computed tomography, MRI magnetic resonance imaging, BIPSS bilateral inferior petrosal sinus sampling

Differential Diagnosis of ACTH-Independent CS

Patients with overt endogenous hypercortisolism whose plasma ACTH concentrations are consistently lower than 1.1 pmol/l (5 pg/ml) are very likely to have ACTH-independent CS caused by primary adrenal pathology [18, 38]. Conversely, plasma ACTH concentrations persistently greater than 3.3 pmol/l (15 pg/ml) almost invariably point toward ACTH-dependent pathologies (e.g., CD or ectopic ACTH and—very rarely—ectopic CRH syndrome). The gray zone between these two cutoff points (lower than 1.1 pmol/l and greater than 3.3 pmol/l) requires a cautious interpretation, because patients with ACTH independent (adrenal cause) as well as those with CD may have intermediate values [18, 21, 69].

Biochemical Testing and Imaging

A single plasma ACTH value at the lower cutoff point (just above 1.1 pmol/l) may not convincingly exclude adrenal ACTH-independent CS, especially in cases of mild hypercortisolism [38]. Establishing the right diagnosis is essential, because the primary curative therapy for ACTH-independent CS is adrenal surgery. Thus, in cases with equivocal results in which ACTH is not fully suppressed, CRH stimulation test should be performed to help in the differential diagnosis [70]. Blood is drawn at 15 and 0 min prior to CRH for cortisol and ACTH (in a prechilled collecting tube on ice). CRH is then injected at a dose of 1 µg/kg body weight (maximum of

200 µg), and blood samples are collected at 15 and 30 min for ACTH and at 30 and 45 min for cortisol. Patients with adrenal ACTH-independent CS have a diminished or no ACTH response to stimulation with CRH due to negative feedback, while in pituitary ACTH-dependent CS the ACTH response is usually exaggerated. The diagnostic sensitivity and specificity of the CRH test are 93 and 100 %, respectively, when the response of ACTH is more than 35 % and cortisol more than 20 % of baseline [5, 71].

In cases in which the biochemical diagnosis of ACTH-independent CS has been established, attention can be turned to imaging the adrenal gland with computed tomography (CT) or magnetic resonance imaging (MRI) to lateralize the source of cortisol hypersecretion [72].

Unilateral hyperfunctioning adenoma, together with functionally active adrenocortical carcinoma accounts for the majority of ACTH-independent CS cases. The familial and sporadic BAIMAH and PPNAD are among the less common causes [73]. Unilateral adrenocortical adenoma and carcinoma may have identifiable features on anatomical imaging with CT or MRI. Adenomas are smaller in size than carcinomas (~4 vs. ~14 cm, on average) and have a lower mean unenhanced CT attenuation value (11 vs. 28 Hounsfield units) [74]. Among the other anatomical imaging features favoring adrenocortical carcinoma rather than adenoma are the heterogeneous appearance, presence of necrosis and calcifications, and loco-regional or distant lesions suspicious for

metastatic disease [74]. In cases with suspicious lesion characteristics on CT scan (morphology or large size), ¹⁸ F-fluorodeoxyglucose positron emission tomography (FDG-PET) scan may help in the differential diagnosis with 100% sensitivity and 88% specificity reported [46, 75]. An adrenal to liver maximum standardized uptake value of FDG-PET above 1.45 is highly predictive of adrenocortical carcinoma [75].

Bilateral adrenal pathologies associated with ACTH-independent CS also have characteristic imaging features. Patients with BAIMAH have both glands grossly enlarged, contain nodules larger than 1 cm in diameter (up to 3–4 cm) that are easily visible on CT or MRI. Adrenals of patients with PPNAD usually have multiple small (<1 cm) nodules bilaterally, with no overall glandular enlargement and normal intervening adrenal tissue [74]. However, when these nodules are tiny (<2–5 mm) they are very difficult to recognize and the adrenal glands may appear completely normal. Thus, in an established diagnosis of ACTH-independent endogenous CS with normal adrenal glands on imaging, genetic testing for mutations of *PRKARIA* gene or assessment of other features associated with Carney's complex (e.g., lentigines, myxoma) can help establishing the right diagnosis [18]. In addition, a paradoxical increase in cortisol excretion in response to dexamethasone suppression during the Liddle test can further help identify patients with PPNAD [13, 76]. The test is performed by collecting urine for baseline cortisol excretion (2 separate days) followed by LDDS with 0.5 mg dexamethasone every 6 h for 48 h and high dose dexamethasone suppression (HDDS) with 2 mg every 6 h for another 48 h. The results are interpreted by comparing mean baseline values of urinary cortisol with values on the second day of the high-dosage dexamethasone (day 6 of the test).

Differential Diagnosis of ACTH-Dependent CS

Patients with endogenous hypercortisolism who have plasma ACTH concentrations repeatedly greater than 3.3 pmol/l (15 pg/ml) are very likely to have an ACTH-dependent CS (see Section "Differential Diagnosis of ACTH-Independent CS"). However, differentiating

between pituitary and extra-pituitary causes of ACTH-dependent CS can be a considerable challenge.

Biochemical Testing and Imaging

Although no single best strategy exists to differentiate pituitary from extra-pituitary causes of ACTH-dependent CS, combination of biochemical tests and imaging techniques with careful interpretation of results in light of pretest clinical characteristics is the preferred approach. For example, it has been shown that in 90% of women with consistently elevated plasma ACTH and Cushingoid clinical features the cause of the syndrome is corticotrophin secreting pituitary adenoma (CD) [18]. A pituitary MRI with gadolinium enhancement may be performed in such cases to confirm the diagnosis; however, it has to be noted that, due to their small size, a substantial proportion of pituitary tumors (up to 50%) may remain unrecognized and pose a diagnostic dilemma. Moreover, there is a 10% rate of non-functioning pituitary incidentalomas in the normal population, emphasizing further the need for careful biochemical discrimination of pituitary from extra-pituitary sources of ACTH before establishing a definitive diagnosis [74, 77]. The appearance of adrenal glands on anatomical imaging may give a hint at the possible source, because adrenal glands of patients with ectopic ACTH-dependent CS are virtually always homogeneously enlarged, whereas glands may appear normal in one-third of patients with CD [78].

While one of the first biochemical clues for differentiating pituitary from extra-pituitary ACTH sources is the tendency of ACTH levels to be higher in ectopic ACTH syndrome than in CD, the overlap in ACTH values is such that the magnitude of its increase alone may not be a reliable marker for distinguishing between the two conditions [21]. The overnight or 2 days HDDS tests (2 mg given every 6 h for 48 h or a single 8 mg overnight dose) can be used to further help in the differential diagnosis. This biochemical method is a dynamic noninvasive test based on the relative sensitivity of pituitary ACTH producing adenomas to the negative feedback effect of high supraphysiological glucocorticoid doses

(given orally or intravenously), compared with the resistance usually shown by extra-pituitary tumors. Approximately 80 % of patients with CD will have positive HDDS test demonstrating suppression of serum cortisol to levels less than 50 % of baseline [18]; however, the fact that up to 30 % of patients may have false results must be taken into account when data are interpreted [30, 43, 44].

Most ACTH secreting pituitary adenomas express CRH receptors and retain sensitivity to CRH stimulation, while ectopic ACTH producing tumors are usually resistant to CRH [30, 70]. Because of this biological phenomenon, CRH stimulation test can also be used in differentiating pituitary (CD) from ectopic ACTH-dependent CS cases [70]. CRH is given as an intravenous bolus at a dose of 1 µg/kg (100 µg in adults). The test has 93 % sensitivity and nearly 100 % specificity for CD based on increase of ACTH serum concentrations by at least 35 % of baseline at 15 and 30 min after CRH stimulation and 20 % or more increase in cortisol at 30 and 45 min [71]. Although CRH stimulation tests are very useful in differential diagnosis of ectopic CS, it must be noted that some extra-pituitary ACTH-producing tumors (mostly well-differentiated benign carcinoids) express CRH receptors and may respond to CRH stimulation in the same way as pituitary ACTH-secreting adenomas [5].

The diagnosis of CD can be established with certainty in a patient with proven ACTH-dependent CS (based on elevated serum ACTH levels), positive HDDS and CRH stimulation tests, and an MRI scan revealing a pituitary tumor of 6 mm or larger. However, up to 40 % of patients with biochemically proven CD have normal pituitary MRI scans [18, 69]. In such cases or in patients with discordant noninvasive biochemical tests, bilateral inferior petrosal sinus sampling (BIPSS) should be used to determine the gradient of ACTH from the pituitary gland to the periphery in order to discriminate between pituitary and extra-pituitary ACTH sources [30]. BIPSS is an invasive technique requiring placement of catheters in both inferior petrosal sinuses and determining unstimulated (baseline) and CRH

stimulated (at 3 and 5 min after injection) central-to-peripheral ACTH ratio. When the basal ratio is more than 2:1 or the ratio after CRH stimulation (1 µg/kg or single dose of 100 µg) is more than 3:1, the test results are consistent with CD with a sensitivity of 90–94 % and specificity of 67–94 % [18, 72, 79]. BIPSS is considered by many the best means for differential diagnosis of CD and lateralization of the ACTH source within the pituitary gland, and it has the highest accuracy in children outweighing other imaging techniques [80]. However, the drawback to this otherwise very helpful diagnostic method is that it is an invasive procedure that can only be performed in a major clinical center by a highly experienced interventional radiologist.

In cases in which the biochemical workup and brain imaging has confirmed the lack of a pituitary ACTH secreting adenoma, CT or MRI of the neck, chest, and abdomen should be performed in search for the extra-pituitary source of ACTH. CT or MRI scan combined with ¹¹¹In-pentetetotide scintigraphy (octreoscan) has high detection rate and will localize tumors in 70–90 % of ectopic ACTH-dependent CS [43, 44, 81]. Although standard functional imaging modalities such as octreoscan and FDG-PET may confirm functionality for a lesion seen on anatomical imaging, they have only rarely been shown to reveal occult neoplasms that are not visible on CT and or MRI [18, 82, 83].

The majority of extra-pituitary ACTH secreting tumors that cause ectopic CS are neuroendocrine neoplasms including bronchial or thymic carcinoids, neuroendocrine tumors of the pancreas and gastrointestinal tract (GIPNETs), medullary thyroid cancer, and pheochromocytomas. Unique feature of these neoplasms is the expression of somatostatin receptors by tumor cells that can be targeted by isotope labeled somatostatin analogs [84]. A PET/CT technique using somatostatin analogs labeled with ⁶⁸Gallium isotope (⁶⁸Ga-DOTATATE) has been shown to offer diagnostic advantages over conventional anatomical and functional imaging in patients with GIPNETs [85]. The role and effectiveness of ⁶⁸Ga-DOTATATE in CS population has not yet been clarified. However, it has been recently demonstrated that

⁶⁸Ga-DOTATATE can localize occult neuroendocrine tumors in approximately 30% of symptomatic patients with previously unknown primary neoplasms that offers the opportunity to use ⁶⁸Ga-DOTATATE in patients with occult ectopic ACTH secreting tumors [85].

Unfortunately, despite exhaustive repeated evaluations and prolonged follow-up, the source of ACTH production may remain occult in up to 15% of patients with CS [18, 43].

Management

According to current Endocrine Society's Clinical Practice Guideline, effective management of CS is defined by the normalization of hypercortisolemia (or pathological effects of cortisol), and normalization of comorbidities by directly treating the cause of CS and by adjunctive treatments of the associated medical condition(s) (e.g., antihyperglycemics, antihypertensives) [86]. Surgical resection of the causative tumor(s) is considered the first-line approach. The choices of second-line treatments include medication, and radiation therapy and or bilateral adrenalectomy for pituitary ACTH secreting tumors refractory to other therapies [86]. However, the therapeutic strategies must be individualized to each patient and may vary broadly from permanently curing the disorder by resecting or ablating its cause to merely controlling the hypercortisolism in patients in whom a cure cannot be achieved. Each stage in the treatment should provide maximum probability of cure with the least chance of permanent endocrine deficiency or other undesirable side effects.

Management of ACTH-Dependent CS

Experts recommend that resection of pituitary tumor producing CD or extra-pituitary neoplasm resulting in ectopic ACTH-dependent CS should be the first-line approach unless surgery is unlikely to significantly alleviate hypercortisolism or is not possible [86].

First-Line Therapy for Cushing Disease: Tumor-Specific Surgery

The treatment of choice for patients with CD with a clearly defined microadenoma on MRI is transsphenoidal microadenomectomy. In cases in which the pituitary tumor is not well circumscribed, and future pregnancies are not desired, subtotal resection of the anterior pituitary (85–90%) may be performed. When surgery is performed at experienced, high-volume centers by expert pituitary neurosurgeons, overall long-term remission rates shown in adult patients series average 80% with low surgical morbidity, and mortality rates typically less than 1%; however, late recurrence reduces the permanent cure rate to approximately 60–70% [87–90]. It has been shown that in patients with persistent disease in the early postoperative period, immediate reoperation may be of benefit leading to remission in nearly 70% of these cases [91].

One of the largest prospective studies in children with CD ($n=200$) demonstrated that resection of pituitary adenoma in pediatric patient can be safe, effective, and durable when performed at centers with substantial experience [22]. It has been demonstrated that a very high remission rate (98%) can be achieved after transsphenoidal microadenomectomy in children, and long-term remission has been associated with younger age, smaller adenoma, and absence of signs of invasion [22].

There is no cortisol threshold value that fully excludes possible recurrence after surgery; however, long-term remission is most probable when postoperative concentration of cortisol in serum is less than 1.8 µg/dl (50 nmol/l) [86, 92]. While the overall remission rate on long-term follow-up approximates 60%, patients may suffer deficiencies of other pituitary hormones in up to 50% of cases [90].

Glucocorticoid replacement therapy may be required postoperatively in patients who are hypocortisolemic until the activity of HPA recovers (often for a period of 6–18 months), although patients with mild-to-moderate hypercortisolism preoperatively are less likely to develop severe symptoms of glucocorticoid

insufficiency (Addisonian crisis) [18]. Endocrine Society's Clinical Practice Guideline recommend glucocorticoid replacement with hydrocortisone at doses 10–12 mg/m²/day divided into two intakes daily, with the first dose taken as soon as possible after surgery [86, 93]. Replacement therapy with hydrocortisone is preferred because using more potent synthetic glucocorticoids with a longer half-life may prolong HPA axis recovery [86].

Surgery for Ectopic ACTH-Dependent CS

The optimal first-line therapy for ectopic ACTH-dependent CS is surgical excision of the tumor, aiming at removal of the source of extra-pituitary ACTH secretion and cure of the metabolic disorder. Several series have demonstrated 40–80 % remission rates after surgery for pulmonary carcinoid tumors and nearly 90 % for other localized neuroendocrine tumors [3, 43, 44, 94]. In cases with advanced widely metastatic tumors it is practically impossible to achieve cure solely by surgical resection, and the consequences of hypercortisolism in such cases need to be treated medically or by bilateral adrenalectomy. However, when metastatic disease is limited to the liver and the lesions appear amenable to aggressive treatment, resection or ablation of the metastases after resection of the primary tumor may result in cure [43, 81, 95].

CS caused by ectopic CRH secreting tumors is an exclusively rare disorder and the literature deals only with case reports [3, 96]. The treatment and prognosis of this condition is the same as for ectopic ACTH-dependent CS cases, and the cure rate depends upon the malignancy of the tumor and whether it can be completely resected.

First-Line Therapy for ACTH-Independent CS

The first-line therapeutic approach to patients with primary adrenal diseases is generally directed at removal of the affected adrenal gland(s).

Surgery for Adrenocortical Adenomas

Approximately 10–22 % of CS cases are due to unilateral functional adrenocortical adenomas (see Table 9.2). Unilateral adrenalectomy is the only curative therapy for these patients. Laparoscopic (retroperitoneal or transperitoneal) adrenalectomy has become the gold standard approach for adrenal adenomas, because it is associated with less postoperative morbidity, hospital stay, and cost compared with open adrenalectomy [97, 98]. When performed by experienced endocrine surgeons, unilateral adrenalectomy is curative in nearly 100 % of adults and children with cortisol producing adrenal adenomas [99].

The advent of widespread use of CT and MRI has resulted in an increased discovery of adrenal incidentalomas. Up to 30 % of patients with an adrenal incidentaloma may have subclinical CS with biochemical evidence of abnormal cortisol secretion, but absence of clinical signs and symptoms of CS [32, 35, 36]. Optimal therapeutic approaches in these patients are not well defined, and the role of adrenalectomy is not completely clarified. The few available retrospective and small cohort prospective studies suggest that patients with subclinical CS and metabolic abnormalities are likely to benefit from adrenalectomy [32, 34, 100, 101]. Although data from bigger randomized studies are currently lacking, a large randomized prospective clinical trial comparing outcomes of surgery versus conservative management and follow up in patients with subclinical CS is currently ongoing (ClinicalTrials.gov Identifier: NCT02001051).

In patients with ACTH-independent CS due to functional adrenal adenomas, recovery of normal function of suppressed hypothalamus, pituitary, and contralateral adrenal gland may be delayed. Therefore, the patients often require glucocorticoid replacement therapy for several months after curative adrenalectomy, similar to that for patients with CD after transsphenoidal pituitary microadenoma removal. Generally, patients are given higher than normal glucocorticoid replacement intraoperatively and postoperatively to avoid symptoms and signs of acute steroid

withdrawal. A dose of 100 mg of hydrocortisone every 8 h intravenously may be administered over the first 24-h with the first dose given after induction of anesthesia. A rapid taper follows over a few days until hydrocortisone can be given by mouth. A taper may be achieved by decreasing each successive daily dose to 50 % of the previous day's dose [86].

Recovery of HPA axis can be evaluated by measuring serum cortisol level with blood samples obtained before the morning hydrocortisone dose (every 3 months), followed by an ACTH stimulation test starting when the level is 7.4 µg/dl (200 nmol/l) or more. The axis has recovered if the baseline or stimulated level is approximately 18 µg/dl (500 nmol/l) or greater. Patients with cortisol levels below 5 µg/dl (138 nmol/l) should remain on glucocorticoids until retested in 3–6 months [86].

Patients with CS have a greater (more than tenfold) risk of developing venous thromboembolism, especially if undergoing surgery, and perioperative hypercoagulability prophylaxis should strongly be considered in patients undergoing adrenal surgery (open or laparoscopic).

Surgery for CS Caused by Adrenocortical Carcinoma

Functionally active, cortisol-producing adrenocortical carcinoma is the underlying cause in approximately 15 % of ACTH-independent CS cases [13, 21]. Complete surgical excision of localized adrenocortical carcinoma with locoregional lymphadenectomy in cases with locally advanced disease (R0 resection) is the mainstay of potentially curative approaches [45]. However, more than half of the patients who have undergone complete removal of the tumor are destined to have disease recurrence, often with metastases [46]. In cases of recurrent adrenocortical carcinoma with localized or low tumor burden, a second R0 resection may be effective in improving survival if the time to first recurrence was >12 months [45]. Despite improved survival reported in patients with resectable oligometastatic disease, prognosis in advanced inoperable cases is poor with a 5-year overall survival being <15 % [45, 102].

The aggressive behavior and the high recurrence rate of adrenocortical carcinoma provide the rationale for the use of adjuvant therapy, and mitotane has been the reference drug for decades [45]. According to consensus statement of a panel of international experts, patients with potential residual disease after surgery for adrenocortical carcinoma and/or Ki67 more than 10 % should be offered adjuvant mitotane, whereas adjuvant therapy is not considered mandatory for patients with stage I or II disease, histologically proven R0 resection; and Ki67 expressed in ≤10 % of neoplastic cells [45].

Surgery for ACTH-Independent Bilateral Adrenal Disease

The familial and sporadic forms of bilateral macronodular and micronodular adrenal hyperplasia with its pigmented variant PPNAD are among the less common causes of ACTH-independent CS (see Table 9.2) [3, 18, 30, 73].

Laparoscopic bilateral adrenalectomy is uniformly effective for patients with ACTH-independent CS caused by primary bilateral macronodular or micronodular adrenal hyperplasia. It is considered the gold standard therapeutic approach, and, when performed by experienced endocrine surgeons, is curative in virtually all patients [3, 103–106]. Experts in the field recommend against subtotal or unilateral adrenalectomy in these cases, because of high risk of disease recurrence [30, 86]. Bilateral adrenalectomy will inevitably cause permanent adrenal insufficiency requiring a lifelong mineralocorticoid and glucocorticoid replacement therapy (hydrocortisone 15±3 mg in the morning and 5±2 mg in the evening with fludrocortisone 100 µg/day); however, it will not lead to the development of Nelson's syndrome that is a major concern after bilateral adrenalectomy in patients with CD refractory to other treatments.

Before discharge patients should be switched to 0.5 mg of dexamethasone daily for confirming cure, and UFC and diurnal serum cortisol must be measured 24- and 48-h after dexamethasone initiation. Undetectable serum cortisol (<1 µg/dl) and UFC (<1.3 µg/24-h) is considered biochemical evidence of cure [86].

Prior to discharge, patients and patients' family members should be thoroughly educated on adrenal insufficiency signs and symptoms including instructions on "sick day" dose adjustment and when and how to use emergency hydrocortisone injection. Patients should be provided with medical alert bracelets and hydrocortisone sodium succinate vials for emergency use as described in detail in information prepared by the National Institutes of Health Clinical Center [107].

Second-Line Therapeutic Options

Specific first-line treatments discussed earlier may be delayed during diagnostic testing or while adjustments of second-line therapeutic regimens are made to achieve control of hypercortisolism. During this time, standard clinical practice treatment of associated comorbidities such as diabetes, hypertension, and osteoporosis must be started, continued after remission, and stopped only if the medical conditions have resolved.

Adjunctive Medical Treatment of CS

While CS is optimally treated by surgery, medical or radiotherapy is often required when surgery is unsuccessful, contraindicated, or delayed.

There are three classes of drugs commonly used for medical control of hypercortisolism, including: (1) drugs that inhibit steroidogenesis and have adrenolytic effect such as ketoconazole, metyrapone, mitotane, and etomidate; (2) modulators of ACTH release such as the somatostatin analog pasireotide and dopamine agonist cabergoline; (3) blockers of glucocorticoid receptors such as mifepristone [108].

While one of the most widely used drugs metyrapone and ketoconazole have rapid onset of action, initial control of hypercortisolism in patients with CD can often be lost due to ACTH oversecretion known as "escape phenomenon." Consequently, these drugs are not recommended for long-term sole treatment of CD and can be used either in preparation for or as adjunctive treatment after transsphenoidal pituitary surgery

or radiotherapy [18]. On the other hand, adrenolytic drug mitotane has delayed onset, but long-lasting action without "escape phenomenon." These pharmaceuticals are often used for hypercortisolism control during diagnostic workup of CS and while preparing patients for resection of ACTH secreting neoplasm or waiting for bilateral adrenalectomy in cases with occult ectopic tumors [44, 94]. Intravenous adrenolytic etomidate can be a very useful alternative for acute control of severe hypercortisolism in adults and children when the oral route is not an option [109, 110]. However, these drugs have multiple side effects including hepatotoxicity (ketoconazole, mitotane) gastrointestinal side effects (ketoconazole, metyrapone, mitotane), and nephrotoxicity and sedation (etomidate) [18, 111].

ACTH secreting pituitary tumors may also express dopamine D₂ and somatostatin (5 and 2) receptors that can be targeted by cabergoline and pasireotide, respectively. These treatments have been shown to normalize UFC in up to 40% of patients with CD [13, 112, 113]. Major side effects include gastrointestinal discomfort (cabergoline and pasireotide), hyperglycemia, and cholestasis (pasireotide), as well as headache, dizziness, and cardiac valve fibrosis (cabergoline) [13].

In cases with failed surgery or patients who are not surgical candidates, glucocorticoid and progesterone receptor antagonist mifepristone can be used for control of hyperglycemia and acute psychosis secondary to hypercortisolism. While mifepristone has a rapid onset of action, it may cause hypokalemia, worsening hypertension, endometrial hyperplasia, and gastrointestinal side effects [114].

Pituitary Irradiation

Pituitary irradiation is a valuable treatment option for patients in whom transsphenoidal adenoma resection has failed or tumor is not well circumscribed and future pregnancies are desired in which case subtotal resection of the anterior pituitary is not an option.

Conventional fractionated photon beam radiotherapy may result in remission in up to 83 and

78% of patients in adult and pediatric series, respectively; however, it is associated with long-term hypopituitarism, and results can be significantly delayed from 6 to 60 months after treatment [115–117].

Another method for an extremely precise tumor irradiation is stereotactic delivery of radiation through many ports by using computer-assisted planning combined with MRI in order to minimize damage to surrounding structures. Although experience with single-dose stereotactic radiotherapy (also known as stereotactic radiosurgery) is limited, this technique provides less irradiation to neuronal tissues and it is more convenient to patients allowing a single treatment day, rather than 6 weeks of therapy with conventional fractionated radiotherapy [118].

Bilateral Adrenalectomy

Bilateral adrenalectomy (laparoscopic or open) is a safe and effective treatment option for patients with CD refractory to other treatments as well as for patients with occult or unresectable ectopic CS in whom rapid and definitive cure of hypercortisolism is necessary or when other therapies have failed [3]. These patients will need lifelong mineralocorticoid and glucocorticoid replacement therapy (see Section “Surgery for ACTH-independent Bilateral Adrenal Disease”).

A very important concern in patients with CD after bilateral adrenalectomy is Nelson’s syndrome that can develop in 21% of patients [86]. Nelson’s syndrome is due to the lack of cortisol negative feedback and is represented by a local pituitary tumor overgrowth with mass effect and extreme ACTH levels causing hyperpigmentation. Although it is not known conclusively whether radiotherapy can decrease development of Nelson’s syndrome, some physicians advocate prophylactic pituitary irradiation to decrease this risk [119]. These patients must have lifelong follow up with clinical examinations for hyperpigmentation, ACTH measurements, and MRI scans [120].

Follow-Up and Prognosis

Long-term follow-up is an essential part of management of patients with CS, and a multidisciplinary team approach should be used to evaluate and monitor for possible recurrence and ensure adequate hormonal replacement. Long-term treatment of physical, metabolic, and neurobehavioral negative consequences of chronic hypercortisolism is required to reduce morbidity, improve health-related quality of life, and reduce the long-term mortality associated with CS [30]. Although biochemical remission is associated with significant improvement or even reversal of clinical symptoms and signs of CS, some features may not completely normalize, and clinicians should share with their patients that recovery may take from several weeks to several years.

Generally, physical features of CS such as central obesity, muscle wasting/weakness, acne, hirsutism, and purple striae improve first and may disappear gradually over a period of several months to a year; however, these features may be persistent in 10–30% of patients [3, 7, 72, 86, 121, 122]. Similarly, metabolic comorbidities including arterial hypertension, diabetes, menstrual irregularities, libido abnormalities, and osteoporosis improve significantly with reduction or even discontinuation of supportive medications in some patients, but could remain unchanged (up to 25% of patients) or increase in severity in others [3, 72, 121, 123, 124]. Neurobehavioral abnormalities such as anxiety, depression, mood change, and memory loss may improve appreciably after cure of CS; however, underlying psychopathology may persist in a significant proportion of patients (10–40%) [3, 72, 121, 125–127].

Although health-related quality of life (HRQOL) is severely impaired in CS, data from published cross-sectional studies using generic QOL and disease-specific CS questionnaires demonstrated improvement in QOL in up to 80–85% of patients in remission compared to those with hypercortisolism, regardless of the cause of CS or treatment used [3, 128–130]. Yet,

many patients (20–25 %), including children and adolescent may report unchanged or worse HRQOL for many years despite remission of hypercortisolism [30, 72].

Improvement in our understanding of CS and development of new management strategies has led to a dramatic improvement in prognosis for patients with this otherwise devastating condition [41, 131]. Cortisol overproduction can readily be controlled by adrenalectomy, adrenolytics, and/or cortisol synthesis inhibitors; therefore, no patient with CS of any cause should succumb to the complications of persistent hypercortisolism. The advent of laparoscopic surgical methods and minimally invasive neurosurgical techniques has decreased perioperative morbidity and mortality for patients with CS and CD, respectively, to a minimum. Nowadays, long-term mortality and prognosis of patients in initial remission after treatment for CS due to benign causes are excellent and approximate that in the general population [131]. However, patients with ectopic ACTH secretion or adrenocortical carcinoma may have a poor prognosis associated with the underlying malignancy.

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Management of Pheochromocytoma and Paraganglioma

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Background

Pheochromocytomas (PHEOs) and paragangliomas (PGLs) are rare catecholamine-secreting neuroendocrine tumors, which remain unrecognized in up to 50 % of the patients [1]. PHEOs are derived from chromaffin cells of the adrenal medulla and account for approximately 80–85 % of the cases whereas PGLs arise from extra-adrenal autonomic ganglia and occur in about 15–20 % of the cases. PGLs develop from either prevertebral and paravertebral sympathetic ganglia located in the mediastinum, abdomen, and pelvis or parasym pathetic ganglia located along the distribution of cranial and vagus nerves in the regions of the head and neck [2, 3]. Sympathetic paragangliomas (sPGLs) are most commonly found in the abdomen, arising from the organ of

Zuckerkandl, located in proximity to the origin of the inferior mesenteric artery [4]. Like most adrenal PHEOs, sPGLs produce substantial amounts of catecholamines in the majority of the cases [2, 3]. In contrast, parasym pathetic paragangliomas (pPGLs) arise from areas of the carotid body, glomus jugulare, tympanicum, and vagale and are frequently biochemically inactive, found to be producing catecholamines in only 4 % of the cases [2, 5–7].

Biochemically active PHEOs and PGLs can be divided into three different phenotypes based on elevations in the types of catecholamines and their respective metabolites. The noradrenergic phenotype comprises of tumors with a predominant production and secretion of norepinephrine and its metabolite normetanephrine. This phenotype is associated with tumors primarily located in extra-adrenal locations, however, has also been reported in approximately half of the patients with adrenal PHEOs [8, 9]. The adrenergic phenotype consists of tumors predominantly producing and secreting epinephrine and its metabolite metanephrine. The third rare group is composed of tumors producing and secreting mainly dopamine and its metabolite 3-methoxytyramine [8, 10]. Tumors of the adrenergic phenotype are primarily located within the adrenal glands whereas tumors with the dopaminergic phenotype are mainly found in extra-adrenal locations, more specifically associated with head and neck PGLs [10, 11].

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With recent advancements in genetic research, our understanding of familial syndromes associated with PHEOs and PGLs and their underlying tumorigenic mechanisms has reshaped significantly. Originally believed to be mainly a sporadic disease, germ line mutations in 12 well-recognized tumor susceptibility genes have been identified to be responsible for approximately 40 % of the cases [12]. Patients with hereditary mutations often present with disease at a younger age and with multifocal lesions [13, 14]. Furthermore, somatic mutations in these susceptibility genes and other predisposing genes are also involved in the pathogenesis of these tumors. Two fundamental molecular pathways classified by their transcriptional signatures govern the development of these tumors by regulating the hypoxia signaling pathway and the kinase signaling pathway (Fig. 10.1). Cluster one comprises of mutations in genes, which are characterized by a (pseudo)hypoxic signature involving stabilization of the hypoxia-inducible factor [15]. Cluster two comprises of mutations in genes, which cause activation of the RAS-RAF-MAPK and PI3K-AKT-mTOR pathways. A summary of clinical characteristics associated with mutations in genes in these two different clusters is presented in Tables 10.1 and 10.2.

The relationship between genetic mutations, secretory phenotypes, and tumor locations is illustrated in Fig. 10.2. Mutations in the predisposing genes regulate the differentiation of tumor progenitor cells, which in turn influences the expression of enzymes involved in the synthesis of catecholamines and the resultant biochemical phenotype [8, 16]. Additionally, the maturity of the tumor progenitor cells governs disease severity and risk of malignancy by regulating apoptosis, proliferation, and migration of poorly differentiated cells [8].

Clinical Presentation

Previously described as a great mimic, the wide range of nonspecific signs and symptoms associated with PHEOs and PGLs makes the diagnosis of these tumors extremely challenging for physicians. The clinical presentation is highly depen-

dent on the predominant catecholamine being produced and secreted by the tumor (Fig. 10.3), which have different affinities for various target adrenoceptors (Table 10.3). The action of circulating catecholamines on these receptors results in a multitude of hemodynamic and metabolic clinical manifestations and complications (Table 10.4). Patients frequently present with hypertension, headaches, palpitations, sweating, and anxiety with other signs and symptoms being less commonly reported (Table 10.5) [2, 17].

Sudden release of catecholamines from the tumor cells results in a typical episode characterized by a massive increase in blood pressure accompanied by a severe headache, diaphoresis, anxiety, nausea, chest or abdominal pain, pallor, and palpitations with or without tachycardia [2, 18, 19]. Patients with PHEOs and PGLs mainly producing epinephrine have a higher frequency of signs and symptoms and present more commonly with these paroxysmal episodes than patients with tumors producing mainly norepinephrine [20]. Epinephrine has a strong potency for β_2 -adrenoceptors on the vasculature, which may result in severe hypotension, particularly postural hypotension and shock [19, 21–23]. Additionally, the metabolic effects of epinephrine result in hyperglycemia and hyperlipidemia [18]. In contrast, patients with norepinephrine-secreting PHEOs and PGLs usually present with sustained hypertension due to the effect of norepinephrine on α_1 -adrenoceptors on the vasculature [18, 24]. The continuous vasoconstriction may lead to acute ischemia or gradual ischemic changes in different organ systems, most notably in the brain, intestine, kidney, skeletal muscle, and eye [24–28].

The risk of developing a hypertensive crisis is significantly increased during diagnostic procedures such as endoscopy, with administration of some intravenous urographic contrasts and induction of anesthesia [19]. Other factors that lead to a sudden increase in blood pressure include an increase in intra-abdominal pressure, manipulation of the tumor, ingestion of tyramine-rich foods and beverages (e.g., wine, certain cheeses, soy sauce, bananas, and chocolate) and use of certain drugs (e.g., histamine, metoclopramide,

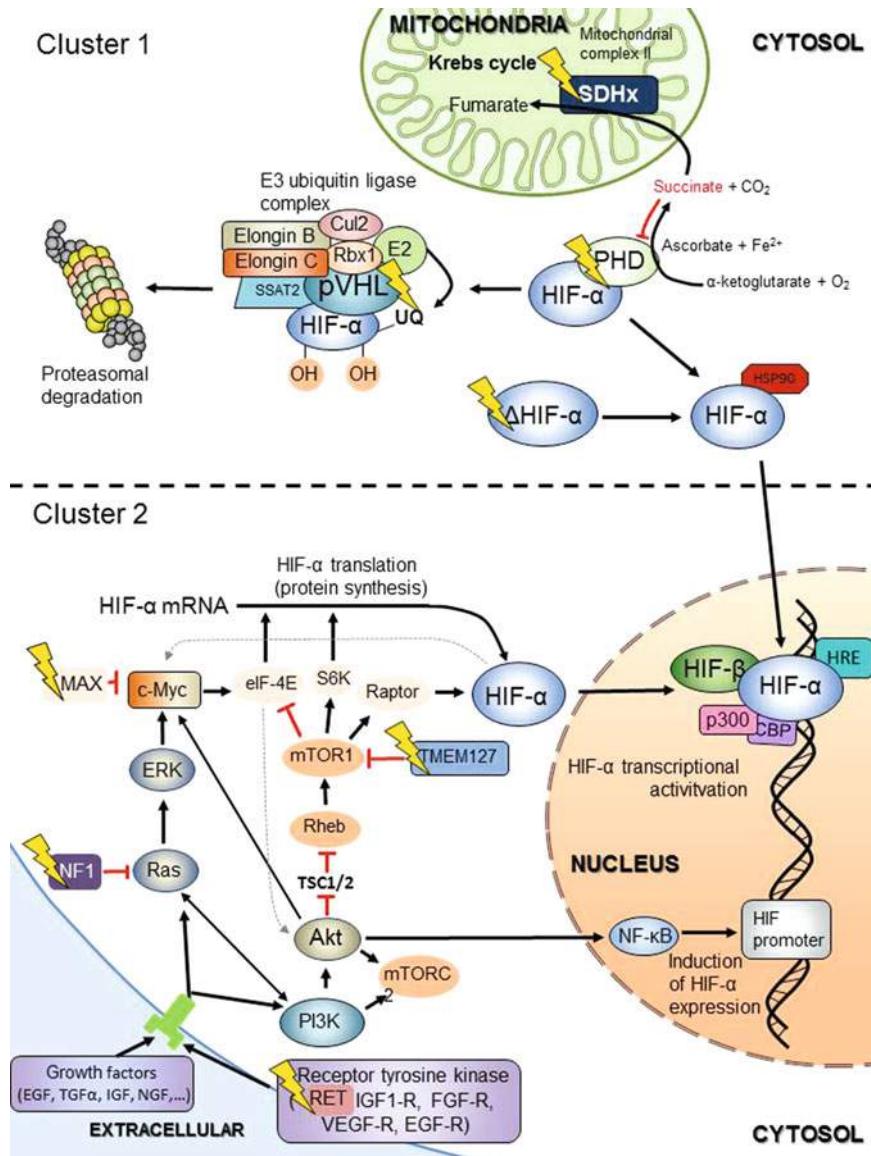


Fig. 10.1 HIF signaling in Cluster 1 and Cluster 2 PHEOs/PGLs. *Akt* RAC-alpha serine/threonine-protein kinase, *CBP* cAMP-response element-binding protein, *c-Myc* Myc proto-oncogene, *Cul2* cullin 2, *E2* E2 ubiquitin-conjugating enzyme, *EGF* epidermal growth factor, *EGF-R* epidermal growth factor receptor, *eIF-4E* eukaryotic translation initiation factor 4E, *ERK* mitogen-activated protein kinase 2, *FGF-R* fibroblast growth factor receptor, *HIF* hypoxia inducible factor, *HRE* hypoxia-responsive elements, *HSP90* heat shock protein 90, *IGF* insulin-like growth factor, *IGF1-R* insulin-like growth factor-1 receptor, *MAX* myc-associated factor X, *mTORC1* mammalian target of rapamycin complex 1, *mTORC2* mammalian target of rapamycin complex 2, *NF1* neurofibromatosis 1, *NF-κB* nuclear factor kappa B, *NGF* nerve growth factor, *p300* histone acetyltransferase p300, *PHD* prolyl hydroxylase domain protein, *PI3K* phosphoinositide 3-kinase, *pVHL* von Hippel-Lindau protein, *Raptor* regulatory associated protein of mTOR, *Ras* rat sarcoma oncogene, *RET* Ret proto-oncogene, *Rbx1* ring box protein 1, *Rheb* Ras homolog enriched in brain, *S6K* S6 kinase, *SDH* succinate dehydrogenase, *SSAT2* spermidine/spermine N1-acetyltransferase 2, *TGFα* transforming growth factor alpha, *TMEM127* transmembrane protein 127, *TSC1/2* tuberous sclerosis complex 1/2, *UQ* ubiquitin, *VEGF-R* vascular endothelial growth factor receptor. Reproduced with permission from ref. [15]

bromin 1, *NF-κB* nuclear factor kappa B, *NGF* nerve growth factor, *p300* histone acetyltransferase p300, *PHD* prolyl hydroxylase domain protein, *PI3K* phosphoinositide 3-kinase, *pVHL* von Hippel-Lindau protein, *Raptor* regulatory associated protein of mTOR, *Ras* rat sarcoma oncogene, *RET* Ret proto-oncogene, *Rbx1* ring box protein 1, *Rheb* Ras homolog enriched in brain, *S6K* S6 kinase, *SDH* succinate dehydrogenase, *SSAT2* spermidine/spermine N1-acetyltransferase 2, *TGFα* transforming growth factor alpha, *TMEM127* transmembrane protein 127, *TSC1/2* tuberous sclerosis complex 1/2, *UQ* ubiquitin, *VEGF-R* vascular endothelial growth factor receptor. Reproduced with permission from ref. [15]

Table 10.1 Cluster 1: PHEO/PGL with (pseudo) hypoxic transcriptional signature

Gene	Syndrome	Mean age		Most common PHEO/ PGL locations	Biochemical profile	Malignancy	Additional clinical features
<i>VHL</i>	VHL	30		Adrenal PHEO NE and DA	NE NE and DA	Low	RCC Hemangioblastoma
<i>SDHAf2</i>	PGL2	30–40		HNPGL	Insufficient data	Insufficient data	
<i>SDHA</i>	PGL4	40		HNPGL sPGL	Insufficient data	Insufficient data	GIST Pituitary adenoma
<i>SDHB</i>	PGL3	30		Adrenal PHEO sPGL	NE NE and DA	High	
<i>SDHC</i>	PGL5	40–50		HNPGL	Non secreting	NE	RCC GIST Pituitary adenoma Pulmonary chondroma
<i>SDHD</i>	PGL1	30–40		HNPGL sPGL	NE NE and DA	Low	RCC GIST Pituitary adenoma Pulmonary chondroma
<i>EPAS1/HIF2A</i>	Pacak-Zhuang syndrome	17–35		sPGL	NE	Moderate	Polycythemia Somatostatinoma Ocular lesions
<i>FH</i>	Reed's syndrome	6–70		Adrenal PHEO sPGL	Insufficient data	Moderate	RCC Leiomyomas
<i>MDH2</i>			Insufficient data	sPGL	Insufficient data	Insufficient data	
<i>SDHB</i>	Carney-Stratakis dyad	23		PGL (may be NS) HNPGL	Insufficient data	Rare	Gastric stromal sarcomas
<i>SDHC</i>				Adrenal PHEO			
<i>SDHD</i>							

DA dopamine, GIST gastrointestinal stromal tumor, HNPGl head and neck paraganglioma, NE norepinephrine, NS non secreting, RCC renal cell carcinoma, sPGL sympathetic paraganglioma

Table 10.2 Cluster 2: PHEO/PGL with kinase receptor signaling pathway

Gene	Syndrome	Mean age	Most common PHEO/ PGL locations	Biochemical profile	Malignancy	Additional clinical features
<i>RET</i>	MEN2	30–40	Adrenal PHEO	EPI EPI and NE	Low	MEN2A: Medullary thyroid cancer, primary hyperparathyroidism MEN2B: Medullary thyroid cancer, marfanoid habitus, mucosal ganglioneuromas
<i>NF1</i>		40–45	Adrenal PHEO	EPI EPI and NE	Low	Café au lait spots Neurofibromas Axillary and inguinal skin freckling Optic gliomas Iris hamartomas Sphenoid bone dysplasia
<i>TMEM127</i>	TMEM127	40–45	Adrenal PHEO	EPI NE	Low	RCC
<i>MAX</i>		30–35	Adrenal PHEO	Intermediate	Moderate	Renal oncocytoma
<i>HRAS</i>	HRAS	31–76	Adrenal PHEO	EPI and NE	Low	

EPI epinephrine, NE norepinephrine

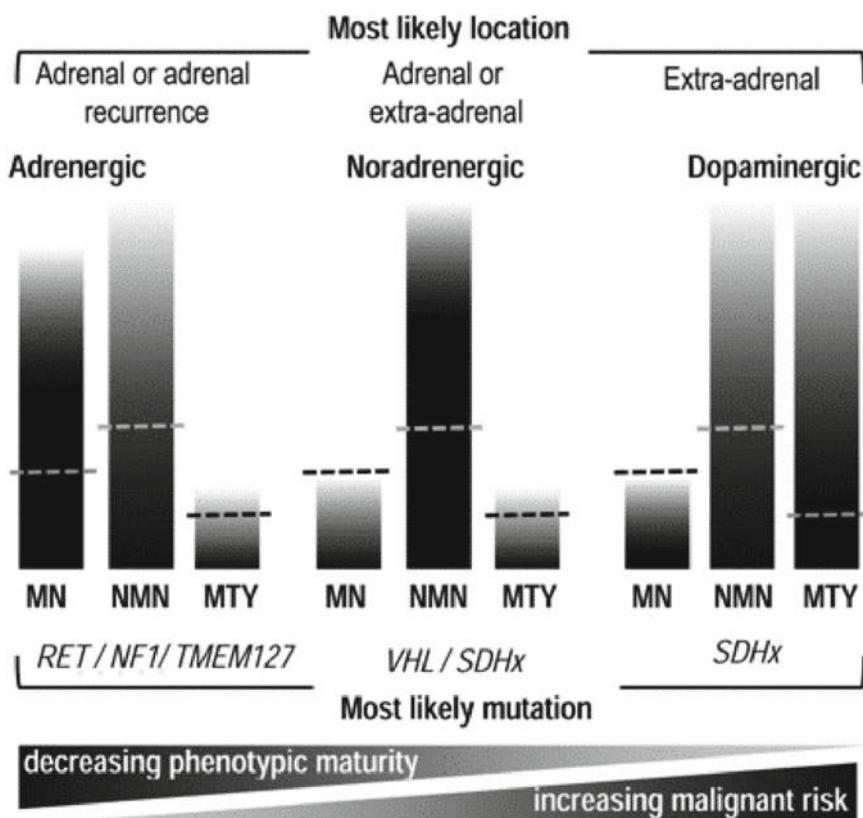


Fig. 10.2 Utility of PHEO/PGL catecholamine phenotypes, as reflected by patterns of increases in plasma normetanephrine (NMN), metanephrine (MN), and

methoxytyramine (MTY), for predicting tumor location, underlying mutation, and malignant risk. Reproduced with permission from ref. [8]

monoamine oxidase inhibitors, tricyclic antidepressants, glucagon, chemotherapeutic agents, and corticosteroids [17, 19, 29]. Patients with PHEOs and PGLs secreting mainly dopamine, present with atypical symptoms such as diarrhea, hypotension, and weight loss [30–32]. Patients frequently complain of compressive symptoms such as pain with abdominal lesions and tinnitus and hearing loss with head and neck PGLs [7, 32, 33]. The action of dopamine on D₂ receptors in the brain may also cause nausea and emesis in these patients [34].

Metastatic PHEO/PGL

There are no reliable markers or histopathological characteristics for accurately distinguishing between benign and malignant PHEO/PGL [14, 35].

The gold standard for diagnosing malignant PHEO/PGL requires evidence of metastatic disease at locations where chromaffin cells are not usually found [35]. Although overall metastatic disease is rare, the risk of developing malignancy in patients followed over a 10-year period is up to 20% [36]. Metachronous metastasis is seen >50% of the patients and can occur even 20 years after the initial diagnosis [37]. Most common sites of metastasis include the local lymph nodes, bone (50%), liver (50%), and lung (30%) [37]. The risk of developing metastasis is higher in patients with sPGLs, particularly those with primary tumors in the infradiaphragmatic paraaortic area and the mediastinum [36].

Although greatly variable, histopathological and immunohistochemical methods that have been designated to identify malignant PHEO/PGL include the Adrenal gland Scaled Score

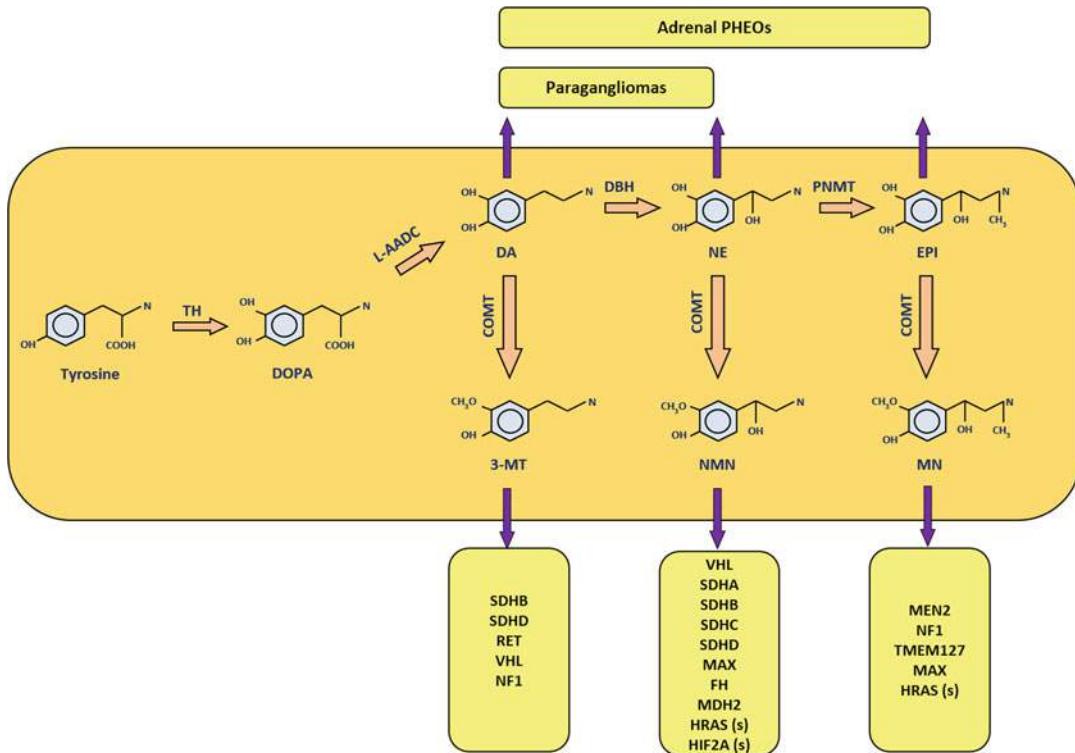


Fig. 10.3 Synthesis and metabolism of catecholamines in chromaffin cells of the adrenal medulla, sympathetic nerves, and extraneuronal tissue. Somatic mutations are denoted by (s). 3-MT 3-methoxytyramine, COMT catechol-O-methyltransferase, DA dopamine, DBH dopamine β -hydroxylase, EPI epinephrine, L-AADC L-amino acid decarboxylase, MN metanephrine, NE norepinephrine, NMN normetanephrine, PNMT phenylethanolamine N-methyltransferase, TH tyrosine hydroxylase

Table 10.3 Catecholamines and adrenoceptors

Name	α_1	α_2	β_1	β_2	D_1 and D_2
Norepinephrine	+++	+++	+++	+//+	0
Epinephrine	++++	++++	++++	+++	0
Dopamine	++/++	?	++++	++	++++

(PASS) and the Ki-67 labeling index. A PASS score of >6 and Ki-67 labeling index >3% suggests a high potential for metastatic behavior [38, 39]. In addition, the presence of a large primary tumor, measuring >5 cm greatly increases the risk of developing metastatic disease [36]. Metastatic PHEOs/PGLs originate from poorly differentiated cells and exhibit deficiencies of enzymes involved in the synthesis of catecholamines. This results in an elevated level of cate-

cholamine precursors DOPA, dopamine and its metabolite 3-methoxytyramine [32, 40, 41]. Even in patients without dopamine elevations, 3-methoxytyramine serves as marker of malignancy and extra-adrenal location, and can be used to monitor disease progression, especially in patients with *SDHB* mutations [32, 40, 41]. Management of metastatic PHEOs/PGLs is aimed at palliating symptoms of catecholamine excess and minimizing disease progression.

Table 10.4 Distribution and function of adrenoceptors

Organ	Component	Receptor	Response	Clinical manifestations and complications
<i>Heart</i>	SA Node	β_1, β_2	Heart rate ++	Angina, palpitations, myocardial infarction,
	Atria	β_1, β_2	Contractility and conduction ++	cardiomyopathies, myocarditis, acute failure, arrhythmias
	AV node	β_1, β_2	Automaticity and conduction +++	
	His-Purkinje	β_1, β_2	Automaticity and conduction +++	
	Ventricles	β_1, β_2 $\alpha_1, \alpha_2; \beta_2$	Contractility, automaticity, conduction, IPM +++	
	Coronary arteries	$\alpha_1, \alpha_2; \beta_2$	Constriction +; dilation ++	
	Arterial supply	α_1	Constriction +	
<i>Lung</i>	Vasculature	$\alpha_1; \beta_2$	Constriction +; dilation ++	Transient ischemic episode, stroke, encephalopathy
<i>Brain</i>	Systemic	$\alpha_1, \alpha_2; \beta_2$	Constriction +; dilation ++	Edema, acute respiratory distress syndrome, pulmonary
	Radial muscle, iris	α_1	Contraction ++	hypertension and fibrosis
	Ciliary muscle	β_2	Relaxation +	
<i>Stomach</i>	Smooth muscle	$\alpha_1, \alpha_2; \beta_2$	Decreased motility and tone +	Orthostatic hypotension
<i>Intestine</i>	Arterial supply	$\alpha_1; \beta_2$	Constriction +++; dilation +	Blurred vision, retinopathy, neuropathy, acute blindness
<i>Gallbladder</i>	Smooth muscle	$\alpha_1, \alpha_2; \beta_1, \beta_2$	Decreased motility and tone +	
	Smooth muscle	β_2	Relaxation +	
	Detrusor	β_2	Relaxation +	Early satiety
<i>Urinary bladder</i>	Trigone and sphincter	α_1	Contraction ++	Constipation, early satiety, intestinal pseudoobstruction,
<i>Skin</i>	Arterioles	α_1, α_2	Constriction +++	ulceration, perforation, ischemia, necrosis
	Sweat glands	α_1	Secretion +	Gallstones
	Pancreas	α_2 β_2	Decreased insulin secretion +++ Increased insulin secretion +	Urinary retention
<i>Kidney</i>	Arterioles	$\alpha_1, \alpha_2; \beta_1, \beta_2$	Constriction ++; dilation ++	Pallor, sweating
<i>Skeletal muscle</i>	Juxtaglomerular cells	β_1	Increased renin secretion	
	Arterioles	$\alpha_1, \alpha_2; \beta_2$	Constriction ++; dilation ++	
	Islet (β cells)	α_2 β_2	Decreased insulin secretion +++ Increased insulin secretion +	
<i>Adipose tissue</i>	—			Hypoglycemia, glycosuria
	—			
	Liver	—		
<i>IPM</i> idioventricular pacemakers				
Hypertension, hyperglycemia, glycosuria, rhabdomyolysis				
Hypertension, hyperglycemia, glycosuria				

Table 10.5 Signs and symptoms of pheochromocytoma and paraganglioma

Signs		Symptoms	
Hypertension	++++	Headaches	++++
Sustained hypertension	++	Palpitations	++++
Paroxysmal hypertension	++	Anxiety/nervousness	+++
Excessive sweating	++++	Tremulousness	++
Tachycardia or reflex bradycardia	+++	Weakness, fatigue	++
Pallor	++	Nausea/vomiting	+
Fasting hyperglycemia	++	Chest pain	+
Postural hypotension	+	Abdominal pain	+
Flushing	+	Dizziness	+
Weight loss	+	Paresthesias	+
Decreased gastrointestinal motility	+	Constipation (rarely diarrhea)	+
Increased respiratory rate	+	Visual disturbances	+

Frequency: highest (++++) to lowest (+). Adapted with permission from ref. [17]

Diagnosis and Localization

Prompt diagnosis of PHEO/PGL is essential in order to prevent serious and potentially lethal complications caused by excessive levels of circulating catecholamines [42–44]. Furthermore, the diagnosis of an underlying genetic mutation, if present, has a pivotal role in the management of the tumor and other associated syndromic features for an overall improved prognosis. Once there is a clinical suspicion of PHEO/PGL, the next immediate step should be to screen the patient by measuring plasma free metanephrenes or urinary fractionated metanephrenes to establish a biochemical diagnosis. This biochemical method has the highest sensitivity (97–100 %) among other tests, which can be attributed to the constant intra-tumoral metabolism of catecholamines and independent steady secretion of these metabolites into the circulation [8, 44–49]. Recent evidence suggests that 3-methoxytyramine, in addition to being a biomarker for accurately diagnosing dopamine-producing tumors, also serves as an indicator of malignancy [8, 10, 11, 40, 50]. Furthermore, in patients with biochemically silent tumors, measurements of chromogranin A aid in the biochemical diagnosis of the tumor [51–53]. There is a strong positive correlation between biochemical levels and tumor size, and therefore, obtaining accurate values can be helpful in monitoring disease progression and

response to treatment. Additionally, determining the biochemical phenotype of the tumor can be helpful in guiding the order of genetic screening and treatment strategy, particularly in determining the appropriate adrenoceptor blockade [41].

In majority of the patients, particularly those with typical clinical manifestations of PHEO/PGL, biochemical levels will usually be highly elevated and the diagnostic workup will proceed to anatomical (CT and MRI) and functional imaging (nuclear medicine) studies. However, in patients with indeterminate results, additional testing with a clonidine suppression test should be performed once all interfering medications have been ruled out [54–56]. While most common causes of drug related false positive results include acetaminophen, L-dopa, buspirone, α -methylldopa, sulfasalazine and mesalamine, phenoxybenzamine, and tricyclic antidepressants are also associated with increases in plasma norepinephrine and normetanephrine levels [54]. Other medications that cause false elevations on biochemical screening include calcium channel blockers, beta blockers, and sympathomimetics [54]. In majority of the cases, it is not feasible to discontinue all interfering medications to maintain patient safety. Therefore, it is more practical and advisable to test while continuing crucial medications and repeat testing or perform clonidine testing when the results seem equivocal. The highest diagnostic sensitivity of the clonidine

suppression test is achieved when responses of plasma normetanephrine as the biomarker are measured [54]. With these tests combined, false positive elevations in plasma normetanephrine levels can be accurately identified [54].

Localization of the PHEO/PGL should be initiated only after a biochemical diagnosis has been established [48, 57]. Imaging may be justified in patients with negative biochemical results when there is a strong family history, a known mutation in one the predisposing genes, a known history of disease or other compelling evidence to suggest the presence of a biochemically silent tumor. According to expert recommendations, optimal results are achieved by performing two imaging modalities, an anatomical imaging study combined with a functional imaging study [48, 58]. Although CT and MRI have been reported to have similar diagnostic sensitivities [2, 58, 59], CT is recommended as the initial imaging modality due to its superior spatial resolution in detecting thoracic, abdominal, and pelvic lesions [57]. MRI is preferred in patients with head and neck PGLs, metastatic disease, CT-contrast allergies and patients in whom radiation exposure is contraindicated [2, 57, 58].

Functional imaging studies typically used for the assessment of PHEOs/PGLs include ¹²³I-metaiodobenzylguanidine (MIBG) scintigraphy, somatostatin receptor scintigraphy (SRS) with ¹¹¹In-pentetetotide (Octreoscan), ¹⁸F-fluorodeoxyglucose(FDG), ¹⁸F-fluorodihydroxyphenyl-alanine (FDOPA), ¹⁸F-fluorodopamine (FDA), and ⁶⁸Ga-DOTA(0)-Tyr(3)-octreotate (DOTATATE) positron emission tomography (PET) CT [58, 59]. The diagnostic performance of ¹²³I-MIBG scintigraphy is inferior to Octreoscan, FDG, FDOPA, and FDA PET CT for the detection of metastatic lesions [60–62]. Therefore, the use of ¹²³I-MIBG scintigraphy as a functional imaging modality is recommended for adrenal PHEOs and for metastatic disease when radiotherapy with ¹³¹I-MIBG is being considered [57]. ¹⁸F-DOPA PET CT is an extremely sensitive and a superior functional imaging method for the detection of head and neck paragangliomas and biochemically silent PHEO/PGL [63, 64]. Additionally, given its competent performance in patients with

SDH mutations, it can serve as a screening imaging study for patients with mutations in the SDHD gene, predisposed to developing head and neck paragangliomas [64, 65]. On the other hand, ¹⁸F-DOPA PET CT is associated with high rates of false negative results in patients harboring *SDH* mutations and adrenal PHEO or sPGL [66]. These results suggest a higher performance and utility of this imaging technique in patients with nonhereditary metastatic disease. Although not widely available, recent evidence has shown a superior performance of ⁶⁸Ga-DOTATATE PET CT compared to not only anatomical but also other functional imaging modalities including FDG, FDOPA, and FDA PET CT in the localization of metastatic lesions in patients with both sporadic and genetic disease, particularly in patients with mutations in the SDHB gene [67–70]. Additionally, it has a higher lesion detection rate in patients with head and neck paragangliomas [71] (Fig. 10.4). Octreoscan and ⁶⁸Ga-DOTATATE PET CT are also particularly helpful in evaluating patients for therapy with somatostatin analogs. An algorithm of localization of pheochromocytoma is shown in Fig. 10.5.

Surgical Management

Management of PHEO/PGL requires a multidisciplinary approach consisting of specialists in the field of endocrinology, oncology, anesthesiology, intensive care, and surgery, who have significant experience in the treatment of patients with these tumors. With surgical resection being the only available curative treatment modality, the primary objective of this intervention is complete removal of the tumor from the primary location as well as from local and distant metastatic sites. For adrenal PHEOs, the current standard of care is minimally invasive surgery, which includes the transperitoneal laparoscopic adrenalectomy or alternatively, the retroperitoneoscopic adrenalectomy [72–78]. In addition, laparoscopic surgery has also been successfully performed in patients with intra-abdominal PGLs, with outcomes comparable to adrenal resection [79]. The laparoscopic approach is associated with lower

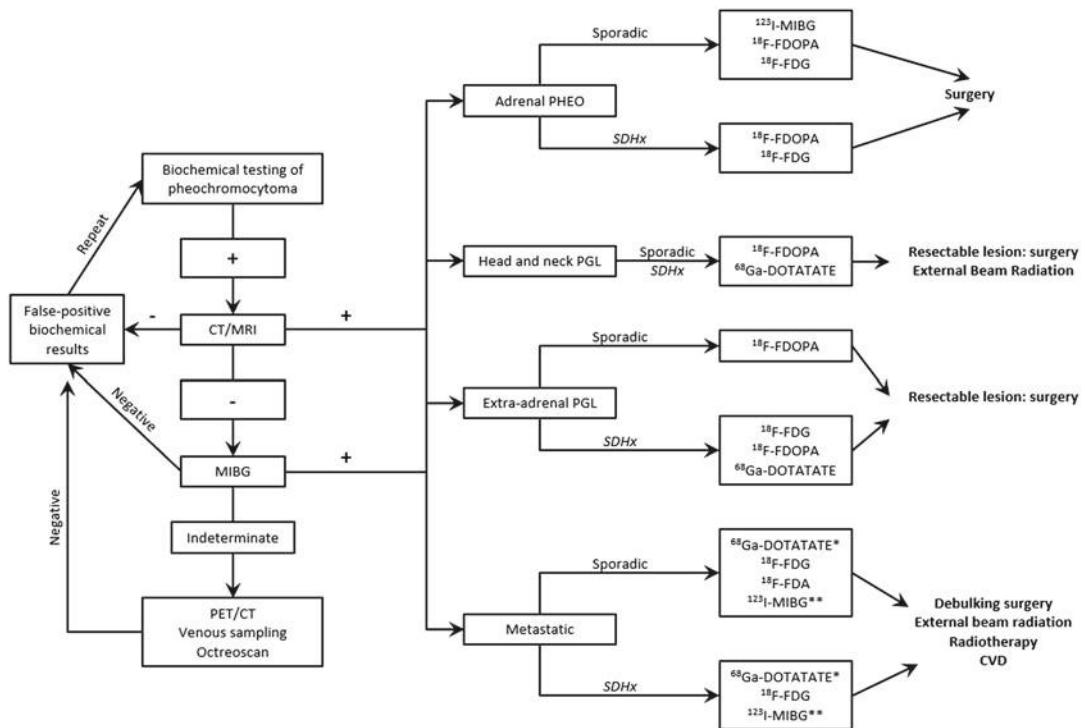


Fig. 10.4 Recommended algorithm for localization of disease in patients with pheochromocytoma and paraganglioma. *Particularly in patients considering peptide receptor radionuclide therapy with DOTA peptides. ** Particularly in patients considering radiotherapy with ^{131}I -MIBG

morbidity, less postoperative pain, and a faster recovery compared to an open procedure [75, 78, 80]. However, caution should be exercised as the insufflation of carbon dioxide to produce a pneumoperitoneum is associated with iatrogenic acidosis and mechanical compression, which causes catecholamine release from the tumor, possibly leading to acute hemodynamic changes, as is seen with tumor manipulation [80].

While the current endocrine society guidelines recommend an open approach in patients with tumors measuring ≥ 6 cm, laparoscopic surgery has also been performed in these patients with variable outcomes [57]. The operative time of the laparoscopic approach and the conversion rate to an open procedure for resection of large tumors depends on the surgeon's experience and the "learning curve" [74]. Laparoscopic surgery, if considered feasible for large tumors, should be performed with great care in order to prevent profuse bleeding, the disruption of the tumor capsule

leading to intraoperative dissemination, peritoneal seeding and recurrence of the tumor [74, 81]. Additionally, an increased amount of tumor manipulation may be needed when resecting a large tumor with the laparoscopic approach causing more pronounced hemodynamic instability [82]. Early careful ligation of the adrenal venous drainage is imminent in preventing massive deposition of stored catecholamines into the systemic circulation, which decreases the frequency of cardiovascular and cerebrovascular complications [82]. Single-port robotic adrenalectomy has been reported as a safe and efficacious method for both complete and partial resection of PHEO with similar success rates, decreased postoperative pain and shorter hospitalization but higher costs compared to laparoscopic and open surgery [83–85].

Cortical sparing adrenalectomy, usually performed as a laparoscopic procedure, is indicated in patients with sporadic and hereditary bilateral

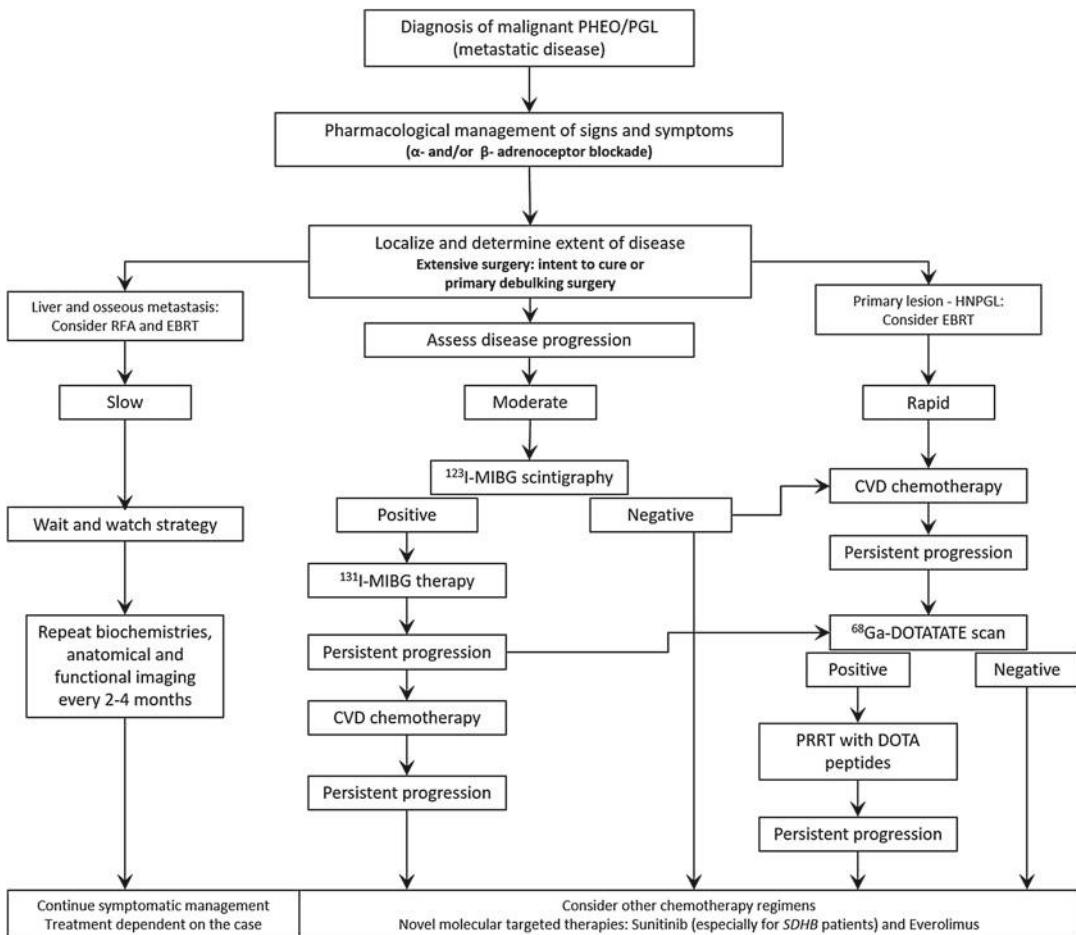


Fig. 10.5 Recommended treatment algorithm for patients with metastatic disease

adrenal PHEOs and in patients with small tumors who have undergone a complete resection of the contralateral adrenal gland [57, 86]. Patients with multiple endocrine neoplasia type 2 and von Hippel–Lindau syndrome presenting with unilateral PHEO have a high risk of developing asynchronous disease in the contralateral gland, necessitating partial adrenalectomy to be performed at the first diagnosis [2, 86, 87]. The risk of recurrence in these patients, reported to be between 0 and 21 %, is due to residual medullary tissue [88–91]. In these cases of recurrent PHEO, repeat laparoscopic partial adrenalectomy has been successfully performed and should be considered, when feasible [92]. The cortical sparing effect of partial adrenalectomy causes retention of normal glucocorticoid function in >50 % of the

patients and is critical in preventing chronic steroid dependence [86, 88–91]. While the most common adverse effects of chronic steroid use include premature osteoporosis, diabetes, and hypertension, insufficient steroid replacement can lead to Addisonian crisis and death.

In patients with metastatic disease, surgery can be performed for either non-curative debulking or for aggressive resection with the goal of complete remission (Fig. 10.5). In some cases, however, surgical intervention represents the only form of palliative treatment modality that can be used for immediate risk reduction and resection of the lesions affecting crucial structures [47]. Extensive surgery for metastatic PHEO/PGL produces successful outcomes and remission in patients with metastasis limited to the abdomen [93, 94]. This

can be facilitated by the intraoperative use of gamma probes labeled with radiopharmaceuticals, particularly ^{123}I -MIBG, which has shown promising results in identification of metastatic sites missed on anatomical imaging [95, 96]. In addition, radio-guided surgery with ^{68}Ga -DOTATATE is being successfully used in patients with metastatic neuroendocrine tumors, including PHEO/PGL and allows for accurate identification of metastatic lesions as small as 0.5 cm [97]. The debulking technique yields variable long-term outcomes in patients with widespread metastatic disease and is primarily based on symptom alleviation [93]. While for patients with exclusively intra-abdominal disease, an aggressive operative approach with intent to treat is justifiable; debulking procedures for extra-abdominal metastases should be aimed at palliating patient symptoms [93]. In addition, it significantly mitigates the tumor burden, which can be critical in improving the efficacy of adjuvant therapies [47, 98].

Preoperative Preparation and Symptomatic Management

Induction of anesthesia, insufflation of the peritoneal cavity and tumor manipulation can induce an intraoperative catecholamine storm leading to life-threatening hypertensive crisis and arrhythmias [80, 99]. Therefore, preoperative optimization of the hemodynamic status with normalization of the blood pressure and heart rate is pivotal in preventing these catastrophic cardiovascular consequences, resulting in a significantly decreased surgical mortality [17]. The length of the preparatory period depends on several components, including the severity of the presentation, associated complications, and the patient's overall health status. In addition to baseline laboratory testing including a complete blood count, a comprehensive metabolic panel and comparative preoperative plasma and urinary catecholamines, metanephrines, and 3-methoxytyramine, a thorough cardiovascular evaluation is also recommended [100]. While electrocardiography can identify hypertrophy, arrhythmia, ischemia, and cardiomyopathy, a Doppler echocardiogram

can assess ventricular systolic and diastolic function and has been reported to be a better predictor of perioperative cardiovascular collapse compared to conventional echocardiography [17, 101]. Abnormalities diagnosed on these tests require additional medical intervention and careful intraoperative monitoring and can prove hazardous, if not identified preoperatively. In addition, discontinuation of smoking and alcohol consumption, and restriction of strenuous physical activity is recommended to prevent complications resulting from significant catecholamine secretion from the tumor [17]. Furthermore, vaccination against encapsulated organisms including pneumococcus, *Haemophilus influenzae*, and meningococcus should be provided to patients with splenic invasive or metastasis who require a splenectomy [17].

Preoperative medical preparation is conventionally initiated with alpha adrenoceptor blockade accompanied by adjunctive pharmacotherapy consisting of calcium channel blockers, beta blockers, and tyrosine hydroxylase inhibitors to achieve hemodynamic stabilization (Table 10.6) [17]. Alternatively, treatment can be initiated with calcium channel blockers followed by tyrosine hydroxylase inhibitors [17]. There are no randomized controlled studies supporting the start time of the adrenoceptor blockade and target values of cardiovascular parameters. However, most institutions initiate blockade 7–14 days prior to surgery with a target blood pressure of less than 130/80 mmHg while seated and systolic greater than 90 mmHg while standing and a target heart rate of 60–70 bpm seated and 70–80 bpm standing [17, 57]. In addition, all patients should be receiving adequate preoperative hydration with intravenous fluids for normalization of the blood volume, which significantly minimizes the risk of developing profound hypotension or shock from sudden diffuse vasodilation after tumor resection [17, 99].

Appropriate administration of preoperative alpha adrenoceptor blockade significantly reduces the risk of perioperative complications, illustrated by a study in which 69% of the patients without preoperative blockade developed complications, compared to only 3% with blockade [102]. Alpha

Table 10.6 Pharmacologic protocol for preoperative management

Drug	Characteristics	Doses	Recommended use	Adverse effects
<i>α-Adrenoceptor blockers (begin 7–14 days prior to surgery)</i>				
<i>Phenoxybenzamine (Dibenzyline)</i>	Nonselective, long-acting and noncompetitive	Oral: 10 mg twice daily → titrated to 1 mg/kg/day IV: 0.5 mg/kg/day for 5 h/day (3 days prior to surgery) 2–5 mg (2–3 times/day) 2–5 mg/day 2–8 mg/day	First line therapy—normalizes BP and expands intravascular volume Withhold at least 12 h before surgery <ul style="list-style-type: none"> For patients with mild hypertension and who cannot tolerate phenoxybenzamine Titrated up slowly to achieve normotension May need to be given the morning of surgery 	Postural hypotension, reflex tachycardia, dizziness, syncope, nasal congestion, edema, postoperative hypotension First-dose postural hypotension
<i>Prazosin (Minipress)</i>	Selective, short-acting and competitive			
<i>Terozolin (Hytrin)</i>	Selective, short-acting and competitive			
<i>Doxazosin (Cardura)</i>	Selective, short-acting and competitive			
<i>β-Adrenoceptor receptor blockers (for tachycardia)</i>				
<i>Propranolol (Inderal)</i>	Nonselective	20–80 mg (1–3 times/day)	<ul style="list-style-type: none"> Help control blood pressure and tachyarrhythmia induced by catecholamines and alpha blockers Initiate at least 3–4 days after alpha blocker 	Use with caution in patients with airway and vascular disease Use with caution in patients with cardiomyopathy
<i>Metoprolol (Lopressor)</i>	Cardioselective	25–50 mg (3–4 times/day)		
<i>Atenolol (Tenormin)</i>	Cardioselective	12.5–25 mg (2–3 times/day)		
<i>Calcium channel blockers (for additional blood pressure control)</i>				
<i>Amlodipine (Norvasc)</i>	Extended-release action	10–20 mg/day	<ul style="list-style-type: none"> Reduces catecholamine mediated calcium influx into vascular smooth muscle 	Headache, flushing, constipation, edema
<i>Nicardipine (Cardene)</i>	Extended-release action	60–90 mg/day		
<i>Nifedipine (Adalat)</i>		30–90 mg/day	<ul style="list-style-type: none"> Monotherapy for patients intolerant of alpha blockers or with mild hypertension 	
<i>Verapamil (Covera-HS and Calan-SR)</i>		180–540 mg/day	<ul style="list-style-type: none"> Beneficial in cardiomyopathy or coronary vasospasm 	
<i>Catecholamine synthesis inhibitor (for additional blood pressure control)</i>				
<i>Metyrosine (Demser)</i>	Competitive inhibitor of tyrosine hydroxylase	250 mg every 8–12 h for a total dose of 1.5–2 g/day	<ul style="list-style-type: none"> Adjunctive therapy for patients with metastatic disease or very hyperactive tumors 	Sedation, depression, anxiety, galactorrhea, extrapyramidal signs, diarrhea, crystalluria

adrenoceptor blockade can be achieved with non-selective and noncompetitive α -adrenoceptor antagonist phenoxybenzamine or with competitive and selective α_1 -blockers including doxazosin, prazosin, and terazosin. In addition to sufficiently normalizing blood pressure, alpha blockade helps in restoration and expansion of the intravascular volume, which is especially critical in patients with refractory hypertension and cardiomyopathy caused by high levels of circulating catecholamines [100]. Alpha antagonists should be initiated with caution and the doses should be titrated up slowly to achieve normotension with clinically tolerable orthostatic hypotension [17, 100]. The more frequently used agent is phenoxybenzamine, which is an irreversible blocker with a long lasting effect, subsiding only after de novo synthesis of the receptor protein [17]. Short acting α_1 -adrenoceptor antagonists have been reported to be safe alternatives, with a similar efficacy of controlling blood pressure compared to phenoxybenzamine [103, 104]. However, these agents are not typically used alone for preoperative preparation and are recommended for patients with unresectable tumors or metastatic requiring long-term symptomatic management, because they are less frequently associated with severe side effects of alpha adrenoceptor blockade [99, 104]. Another approach is to initiate alpha adrenoceptor blockade with phenoxybenzamine and to replace with short acting α_1 -adrenoceptor antagonists closer to surgery to reduce the risk of developing postoperative hypotension [17, 99, 105]. In these cases, due to the short half-life of α_1 -adrenoceptor antagonists, it is critical to also administer them in the morning before surgery [17].

After alpha adrenoceptor blockade has been successfully established on day 2 or 3 of therapy with adequately controlled blood pressure, patients should be advised to add plentiful amount of salt to their diet to restore the contracted intravascular volume and improve postural hypotension [17]. At around the same time, beta adrenoceptor blockade may also be initiated to control tachycardia and tachyarrhythmia induced by catecholamines or alpha adrenoceptor blockers. Beta blockers should never be adminis-

tered prior to alpha blockers due to the risk of exacerbating a hypertensive crisis resulting from the unopposed action of catecholamines on α -adrenoceptors [17]. Additionally, the use of beta blockers in patients with catecholamine-induced cardiomyopathy is contraindicated as it may lead to life-threatening consequences such as severe hypotension, bradycardia and asystolic arrest [106]. Although there is no evidence to suggest a difference in efficacy of cardioselective and nonselective beta adrenoceptor blockers, the former may be preferred in patients with a history of obstructive airway disease and peripheral vascular disease [107].

Alpha adrenoceptor blockers can be supplemented with calcium channel blockers for additional blood pressure control, especially in patients with persistent hypertension in whom increasing the doses of alpha adrenoceptor blockers is not warranted. Additionally, calcium channel blockers can be valuable in the management of patients with adverse reactions to alpha adrenoceptor blockers, particularly orthostatic hypotension, which is not associated with these agents. Patients with only mild or intermittent hypertension can benefit and be adequately controlled on monotherapy with calcium channel blockers instead of alpha adrenoceptor blockers [17, 108].

In some patients, further blood pressure control and symptomatic management is warranted with the addition of metyrosine (Demser), an analog of tyrosine, which competitively inhibits tyrosine hydroxylase thus significantly decreasing the production of catecholamines [17]. This drug is particularly effective for preoperative management of patients with extensive metastatic disease who have persistent hypertension. Also, it is effective in the management of intractable signs and symptoms in patients with exceptionally high levels of catecholamines [17, 109–111]. Using metyrosine preoperatively is associated with decreased intraoperative hemodynamic lability and a reduced rate of postoperative cardiovascular complications [109, 112]. Although it leads to a significant depletion of catecholamine stores after about 3 days of treatment, there is still a substantial amount of circulating catecholamines that can precipitate

serious consequences [113]. Regardless of the dose, there will not be complete depletion of catecholamine stores. Therefore, optimal effects of treatment with this drug are achieved when it is combined with alpha adrenoceptor blockers [3, 17, 109]. Metyrosine is associated with side effects at high doses and should be discontinued if they persist after the dose has been lowered [17, 114].

The utility of appropriate blockade is not only for optimal preparation for surgery but also to prevent life-threatening hypertensive crises in all patients with PHEO/PGL. All patients, regardless of the therapeutic strategy should be appropriately blocked for not only symptomatic management but also to prevent catastrophic consequences. Hypertensive crises can manifest as severe headache, visual disturbances, cerebrovascular accident, myocardial infarction, and congestive heart failure, necessitating urgent treatment with rapid and short acting antihypertensive medications. A hypertensive crisis can be appropriately managed with phentolamine, administered by either 5 mg boluses or a continuous infusion (100 mg of phentolamine in 500 mL of 5% dextrose in water) until blood pressure is adequately controlled. Alternatively, continuous infusions of sodium nitroprusside (0.5–5.0 μ g/min) or nicardipine (5 mg/h), titrated for blood pressure control, or nifedipine (10 mg orally or sublingually) can be used.

Intraoperative Management

Although proper preoperative management significantly aids in preventing intraoperative events and complications, it does not completely eliminate the risk. Efficient intraoperative communication between the surgeon and the anesthesiologist is critical in the success of the surgery [100, 107]. Typically, intraoperative hemodynamic monitoring is established with an intra-arterial catheter, which allows for continuous and prompt recognition of blood pressure fluctuations [100]. In addition, placement of central venous catheters has become the standard of care, allowing for meticulous monitoring and rapid infusion of vasoactive drugs [99]. The use of pulmonary artery

catheterization and transesophageal echocardiography is beneficial in patients with hemodynamic instability or other high-risk comorbidities including pulmonary hypertension, myocardial disease and cardiomyopathy [115, 116].

Intraoperative hypertensive crisis develops most frequently during surgical manipulation of the tumor but can also be precipitated during endotracheal intubation, induction of general anesthesia and creation of the pneumoperitoneum [80, 99]. Depending on the biochemical profile of the tumor, hemodynamic crisis manifests either as tachyarrhythmia in patients with predominantly epinephrine producing tumors or as severe bradycardia occurring with hypertension. While increasing the depths of anesthesia and paralysis can reduce hemodynamic fluctuations, administration of vasoactive agents is often needed to maintain stability [107]. Urgent treatment with sodium nitroprusside, a highly potent arterio-venodilator, should be instituted immediately when this occurs [117]. Nitroglycerin, which mainly affects capacitance vessels resulting in a decreased preload and phentolamine, a competitive alpha adrenoceptor blocker, have both been successfully used as alternatives to manage intraoperative blood pressure elevations [99, 106, 118]. Although phentolamine is known to precipitate tachycardia, it is usually not seen in patients receiving beta adrenoceptor blockade [99, 107, 117]. These agents have a rapid onset and short duration of action, and are administered as titratable intravenous infusions [99, 100, 106, 107, 117]. In addition, magnesium sulfate, nicardipine, and fenoldopam have been successfully used in resistant cases [119, 120]. Tachyarrhythmia and tachycardia is most suitably managed with esmolol, a short acting beta blocker with rapid onset and lidocaine [121]. Studies using labetalol pretreatment (administered before surgical incision) have markedly reduced the supplemental use of intraoperative sodium nitroprusside in patients with adequate preoperative control [122, 123]. Additionally, they established the safety and efficacy of this regimen in reducing intraoperative hypertensive fluctuations and crises without causing an increased rate of postoperative hypotension compared to patients who did not

receive the pretreatment [122, 123]. Although there is limited evidence to support this underutilized strategy, it serves as a safe and effective alternative pretreatment regimen to achieve intraoperative hemodynamic stability, especially in patients with epinephrine producing tumors who are predisposed to developing intraoperative tachycardia and tachyarrhythmia [122, 123].

Sudden and severe intraoperative hypotension usually follows tumor ligation, due to a significant withdrawal of circulating catecholamines in patients with baseline alpha adrenoceptor blockade, volume contraction and surgical blood loss. While dopamine agonists and norepinephrine are only moderately successfully in controlling intraoperative hypotension due to preexisting alpha blockade, massive volume resuscitation has been shown to be more effective [118]. Typically, prior to tumor ligation, patients are adequately stabilized with administration of large fluid boluses and discontinuation of vasodilators. However, vasopressin, which acts through a non-adrenergic mechanism, has been reported to be efficient in refractory cases [124]. When profound hypotension is resistant to treatment with the above approaches, methylene blue can be considered as a safe treatment option [125].

Postoperative Management and Follow-Up

Following an uneventful and a successful surgical resection, particularly if performed through minimally invasive techniques, majority of the patients do not experience any postoperative complications. However, it is advisable that patients be closely monitored in the intensive care unit, especially those who manifest hemodynamic instability and require ventilator support [100]. In addition, vigilant follow-up of electrolytes, plasma glucose and endocrine studies is recommended to identify and manage any potential issues. Persistent postoperative hypotension stems from significant intraoperative blood loss, inadequate volume resuscitation, or residual alpha adrenoceptor blockade [117]. Intra-abdominal hemorrhage is also a likely

cause, which requires urgent intervention and must be ruled out before considering other differential diagnosis. Conversely, persistent post-operative hypertension results from excess administration of intravenous fluids, unintentional ligation of a renal artery or due to continuous secretion of catecholamines from a residual tumor [99]. Special care is warranted in high-risk patients who have undergone complete bilateral adrenalectomies and need steroid replacement therapy.

Postoperative measurements of plasma or urinary metanephrenes and 3-methoxytyramine should be obtained within about 4–6 weeks to ensure complete resection of the tumor. Alternatively, chromogranin A is measured in patients with normal preoperative levels of metanephrenes [51–53]. Persistent biochemical elevations should be followed by imaging studies, which are usually performed 3 months after surgery to identify and locate any residual disease. Outpatient follow-up should continue for at least 10 years, with yearly laboratory screening for recurrent or new disease [126, 127]. In patients with biochemically silent tumors, imaging studies replace laboratory testing and are performed every 1–2 years [127]. Patients with genetic mutations, large, bilateral, and extra-adrenal tumors, extensive metastatic or recurrent disease require closer monitoring with lifelong follow-up and at least yearly appointments compared to other patients [126–128].

MIBG-Radiotherapy

The diagnostic and therapeutic use of MIBG in patients with PHEO/PGL is based on its structural similarities with norepinephrine, which results in the high uptake of this molecule into the tumor cells. Coupled with beta-emitting isotopes, the radioactive compound enters the tumor cells, causing damage and death [129]. For both children and adult patients with inoperable and moderately progressive metastatic disease who have positive uptake (>1%) on ^{123}I -MIBG scintigraphy, radiotherapy with ^{131}I -MIBG serves as a first line treatment. After treatment with ^{131}I -MIBG radiotherapy, stable disease is achieved in 52% of

the patients and a partial hormonal response in 40% [130]. In cases where the progression seems very rapid, treatment with chemotherapeutic agents is more suitable, as response to this radiotherapy may require multiple treatments administered over several months [131, 132]. Certain medications such as labetolol, tricyclic antidepressants, reserpine, some sympathomimetics, and calcium channel antagonists are known to interfere with the cellular uptake of MIBG and should be discontinued before and for the duration of therapy [133, 134]. In addition, caution should be exercised to prevent the uptake of radioactive iodine in the thyroid, which may result in iatrogenic hypothyroidism [135]. Patients should receive appropriate thyroid blockade with SSKI or alternatively, potassium perchlorate (in patients with iodide allergies), started 24–48 h before treatment and continued 10–15 days post-treatment [136].

In addition to following standard radiation safety precautions, hematopoietic parameters including white blood cell ($>3000/\text{mCL}$) and platelets ($>100 \text{ K/mCL}$) should be tested and be in the acceptable range before therapy can be initiated [136]. Treatment doses may be administered in fixed amounts or calculated based on patient weight, ranging from low (64 to $\leq 200 \text{ mCi}$), intermediate ($\leq 500 \text{ mci}$), and single high doses (12–18 mCi/kg). While low doses are well tolerated and can be administered frequently (4–6 weeks), high doses require a longer interval (up to 6 months) to provide an adequate time to recover from dose-dependent toxicity [137, 138]. Response to therapy is assessed at 3–6 months post-treatment with report of symptomatic improvement, repeat imaging (CT or MRI and ^{123}I -MIBG), and biochemical testing [137, 139].

To date, no studies have performed direct systematic comparisons of treatment with these different regimens and therefore, there is no consensus for an optimal dosing strategy. Treatment regimen with nonmyeloablative intermediate doses leads to earlier responses with only a modest increase in toxicity compared to low doses and has become the preferred and more frequently used therapeutic strategy [140]. Although, treatment with low and intermediate

doses does not usually illicit high objective complete responses, it results in significant clinical benefits from partial responses and improvement in symptoms with minimal side effects [131, 132, 140]. On the other hand, while treatment with high doses results in an improved overall complete response (8%), overall survival (64%), and hormonal response (66%), it results in significant hematologic toxicity, requiring autologous stem-cell rescue in patients who develop prolonged myelosuppression [139]. Other serious toxicities associated with high dose therapy include acute respiratory distress syndrome, bronchiolitis obliterans organizing pneumonia, and myelodysplastic syndrome [139].

Patients with mutations in the *SDHB* gene have been reported to have a greater response to treatment with high doses of ^{131}I -MIBG compared to other patients [139]. Data regarding the utility of low or intermediate dose radiotherapy with ^{131}I -MIBG in *SDHB* patients is lacking. A recent publication reported achieving a complete response and remission in a patient treated with a combined regimen consisting of sunitinib and low dose ^{131}I -MIBG [141]. Sunitinib is a tyrosine kinase inhibitor, which targets and inhibits angiogenic factors, particularly vascular endothelial growth factor (VEGF), has been found be anecdotally effective in patients with mutations in genes that cause up regulation of the hypoxic pathway, including *SDHB* and *VHL* [142–144]. Although further studies are needed, this raises a prospect of combining ^{131}I -MIBG therapy and sunitinib in *SDHB* patients, especially with highly aggressive metastatic disease to achieve a more favorable influence on response and survival.

Somatostatin Analogs

Somatostatin analogs including octreotide and lanreotide have been successfully used in the treatment of various neuroendocrine tumors based on their positive expression of somatostatin receptors (SSRs). After detecting a high expression of SSR-subtype 2A and 3 on PHEO/PGL cells, treatment with these analogs was also adopted for PHEO/PGL patients with positive

octreotide scans [145]. Although initial small-scale studies and case reports provided promising evidence, this was not confirmed by other prospective studies conducted on patients with benign and malignant tumors [146–149]. No significant effect on improvement of symptoms and biochemical markers was noted, suggesting that this treatment modality is of limited value for long-term management of patients with benign or malignant PHEO/PGL [148, 149].

Recently, peptide receptor radionuclide therapy (PRRT) using DOTA peptides (DOTATATE, DOTATOC, and DOTANOC) radiolabeled with lutetium (¹⁷⁷Lu), yttrium (⁹⁰Y), or indium (¹¹¹In) is being increasingly used in patients with positive octreotide scan or ⁶⁸Ga-DOTATATE PET/CT. Studies assessing the efficacy of ⁹⁰Y-DOTATOC in both pediatric and adult patients have been published. Treatment with this radiotherapy resulted in significant symptomatic relief and stable disease in 46% of the patients, which sustained for more than 3 years [150, 151]. In addition, treatment with ¹⁷⁷Lu-DOTATATE also showed promising results, reporting stable disease in 50% of the patients enrolled in the study and tumor regression in 50% of the patients with progressive disease [152]. In other case reports, treatment with ¹⁷⁷Lu-DOTATATE in patients with nonmetastatic mediastinal PGLs and multiple spinal canal and cranial PGLs resulted in stable disease or partial responses in 100% of the patients and 70% reduction of tumor volumes, respectively [153, 154]. Although there is limited data to establish the relevance of this therapeutic modality, positive results from published studies suggest promising avenues for future research. Treatment with ¹⁷⁷Lu-DOTATATE and ⁹⁰Y-DOTATOC should be performed at specialized institutes with medical experts who have significant experience with PHEO/PGL and radiopharmaceuticals.

Radiofrequency Ablation and External Beam Radiation

In patients with inoperable primary tumors or metastasis, treatment with radiofrequency ablation (RFA) or external-beam radiation should be

considered. These treatment modalities are aimed at growth inhibition and control, causing arrested progression, rather than complete elimination of the existing tumor. Percutaneous RFA is a safe and minimally invasive treatment modality used for management of painful metastasis and symptoms related to catecholamine excess. Evidence that this treatment is particularly efficacious in the treatment of osseous and liver lesions, which are successfully ablated without recurrence [155–158]. However, careful preprocedural preparation is critical as there is significant tumor lysis during the procedure resulting in a sudden rise in the level of circulating catecholamines, which causes hemodynamic instability [157].

External beam radiation therapy (EBRT), usually used as an adjunct to chemotherapy and systemic radiotherapy for the treatment of metastatic disease, particularly osseous lesions, is increasingly also being accepted as a first line treatment for head and neck PGLs [159]. Earlier anecdotal evidence showed varying effect on symptomatic control with limited inhibition of disease progression, signifying limited utility of this treatment modality in patients with metastatic disease [160, 161]. Moreover, EBRT was previously known to be only effective at high doses, resulting in serious toxicity of the normal neighboring tissue. However, with the advent of intensity modulated radiation therapy and fractionated stereotactic radiosurgery, patients could be administered high treatment doses without causing tissue toxicity. This was followed by retrospective studies, which showed significant biochemical responses, suggesting its potency for control of local as well as systemic symptoms [159, 162]. Although no considerable radiographic regression is observed and patients may have disease progression in absence of concurrent systemic therapy, EBRT has been established as a safe treatment option [159, 162]. However, caution and dose restriction is necessary as patients enrolled for this treatment usually have extensive disease requiring multiple treatments, which may result in radiation-related complications.

Radiotherapeutic options for the treatment of nonresectable head and neck tumors include the traditional fractionated EBRT or the more recently

adopted, radiosurgery using Gamma Knife, linear accelerator (LINAC), or CyberKnife. Conventional surgical treatment for these tumors is usually extremely limited due to inaccessible locations or extreme adherence to adjacent structures resulting in devastating postoperative morbidity related to cranial nerve dysfunction [163]. While EBRT requires large field sizes, which results in serious complications including xerostomia, osteoradionecrosis, and a risk of induction of secondary malignancies, stereotactic radiosurgery allows delivery of high doses with more precise targeting and a decreased toxicity to surrounding tissue [164]. The eligibility of glomus jugulare tumors for treatment with radiosurgery is based on their well-defined and non-infiltrating borders, minimizing the risk of irradiation of surrounding tissue. A meta-analysis of 19 studies consisting of more than 300 patients showed successful tumor control determined by stabilization or regression of tumor volumes in 97% of the patients with glomus jugulare tumors, who were treated with the three different forms of radiosurgery [165]. More recent studies have also yielded successful outcomes based on symptomatic improvement and tumor progression free survival in up to 100% of the patients [166, 167]. Risk of developing toxicity from this treatment, although minimal, exists and is dependent on the treatment volume and radiation dose [166, 168, 169].

Chemotherapy

In patients with inoperable metastatic disease, systemic chemotherapy mainly serves as a form of palliative treatment, improving patient quality of life. The traditional protocol consisting of treatment with cyclophosphamide, vincristine, and dacarbazine (CVD) has been used extensively since 1985, while the safety and efficacy of other chemotherapy agents is not well established [170]. Typically, treatment is administered in 21 days cycles with IV infusions of cyclophosphamide 750 mg/m^2 body surface area on day 1; vincristine 1.4 mg/m^2 on day 1; and dacarbazine 600 mg/m^2 on day 1 and 2 [170, 171]. This regimen was initially chosen due to its high efficacy

in the treatment of neuroblastoma, also a neuroendocrine tumor [170]. Usually believed to have a superior benefit in patients with highly proliferative metastatic disease, CVD chemotherapy has also been shown to impact patients with slowly growing tumors [172]. In majority of the patients, the effectiveness of CVD chemotherapy is evident within 1–4 months of initiation and the treatment is continued indefinitely in these responders. Although most retrospective studies have reported partial or complete responses for shrinking tumor volumes and decreasing catecholamine levels, there is conflicting evidence to support the role of this treatment in increasing the overall survival [172–175].

While in other malignancies, the presence of metastatic disease at the first diagnosis is negatively associated with overall survival; this notion is not completely supported by studies conducted on PHEO/PGL patients [175]. Interestingly, patients presenting with synchronous metastatic disease have been reported to have an improved overall survival compared to patients with metachronous metastasis [174]. In the same study, adrenal PHEO and female gender were found to be negative prognostic factors for treatment with systemic chemotherapy [174]. Additionally, CVD chemotherapy has been found to be particularly beneficial in patients with mutations in the SDHB gene, causing significant tumor shrinkage in 100% of the patients in the study [176]. With reports of an increased utility of ^{131}I -MIBG radiotherapy in patients with *SDHB*, a combined treatment regimen may result in more favorable responses in this patient population [177]. The most commonly reported adverse effects with this treatment regimen include leukopenia, peripheral neuropathy, gastrointestinal toxicity, and hypotension [172, 173]. In addition, caution should be exercised before administration of this therapy, as it is known to exacerbate a hypertensive crisis in some patients.

There is anecdotal evidence of successful treatment with other chemotherapeutic agents used alone or in combination including temozolamide; cyclophosphamide and methotrexate; ifosfamide; etoposide, carboplatin, vincristine, cyclophosphamide, and doxorubicin; and cisplatin

and 5-fluorouracil. A treatment regimen consisting of cyclophosphamide, doxorubicin, and dacarbazine showed tumor responses lower than CVD chemotherapy [178]. However, monotherapy with temozolomide, a 3-methyl analog of mitozolomide, yields successful results comparable to CVD chemotherapy, especially in patients with mutations in the SDHB gene [179].

In conclusion, systemic chemotherapy is effective in the treatment of PHEO/PGL patients with inoperable and rapidly progressive metastatic disease. In addition, it significantly palliates symptoms and should be used for patients who are not eligible or are refractory to other treatment modalities. Furthermore, it may also serve as a neoadjuvant therapy to improve chances of successful surgical treatment of large tumors [173]. CVD chemotherapy usually requires a long course of treatment and is associated with resistance in patients presenting with recurrent disease who were previously treated with this method [173]. Therefore, the use of this treatment should be considered depending on the clinical characteristics of the patient, especially taking into account its limited effect on overall survival.

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of the adrenal cortex that affect cortisol biosynthesis [1]. CAH due to 21-hydroxylase deficiency (21-OHD) accounts for over 95% of cases and is characterized by cortisol deficiency, with or without aldosterone deficiency, and androgen excess [1]. 21-hydroxylase is encoded by the *CYP21A2* gene, located on chromosome 6 within the human leukocyte antigen (HLA) complex. Uncommon forms of CAH are due to loss-of-function mutations in other genes affecting the cortisol biosynthetic pathway including: *CYP11B1* [11- β (beta)

hydroxylase], *HSD3B2* [3- β (beta) hydroxysteroid dehydrogenase], *CYP17A1* [17- α (alpha) hydroxylase], *POR* (P450 oxidoreductase), *STAR* (steroidogenic acute regulatory protein), or *CYP11A1* (side chain cleavage) [2]. CAH in this chapter will refer to 21-OHD unless otherwise specified.

There is a wide spectrum of clinical severity in CAH. There are two major variants: the classic form (incidence 1:15,000) and the nonclassic form (incidence 1:1000), with good genotype–phenotype correlation [3]. The classic or severe form of CAH due to 21-OHD is characterized by impairment of cortisol synthesis, with or without aldosterone deficiency, and androgen excess whereas the more common nonclassic mild variant is characterized by mostly androgen excess [3]. Diagnosis is based on elevated 17-hydroxyprogesterone (17-OHP) levels and 17-OHP and androstenedione are used as biomarkers of disease control. Classic CAH is treated with glucocorticoid and mineralocorticoid replacement; whereas having the nonclassic form is not an absolute indication for glucocorticoid therapy. Carriers of CAH are asymptomatic, although hormonal differences in the function of the hypothalamic-pituitary-adrenal (HPA) axis have been described with exaggerated adrenocorticotrophic hormone (ACTH) response [4]. The treatment of CAH aims to replace deficient hormones and control androgen excess, while avoiding the adverse effects of exogenous glucocorticoid excess.

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Impaired cortisol synthesis alters the negative feedback of cortisol on the hypothalamus and pituitary and leads to hypersecretion of corticotrophin-releasing hormone (CRH) and ACTH. This continued uninhibited activation of the HPA axis leads to adrenal gland hyperplasia along with overproduction of adrenal androgens and contributes to tumor formation. Patients with CAH have increased prevalence of adrenal tumors, including massive myelolipomas [5–7]. This chapter will review the adrenal tumors commonly seen in patients with CAH and their management.

Adrenal Hyperplasia and Tumor Formation

Congenital adrenal hyperplasia is so named because of the abnormal adrenal histology and hyperplasia observed in the first patients diagnosed with this disease [8, 9]. Progressive hyperplasia of the zona reticularis of the adrenal cortex with advancing age was described in 1940s, with subsequent descriptions of abnormal zona reticularis, zona glomerulosa, and zona fasciculata in CAH patients [8, 10, 11]. Indeed, major structural changes of the adrenal gland of the severely affected salt-wasting CAH patients have since been described with abnormal formation of the adrenal medulla and lack of zonation of the adrenal cortex, most likely due to the altered hormonal milieu in utero (Fig. 11.1) [12]. Owing to the derangements in the HPA axis along with adrenal gland hyperplasia and abnormal development, it has been speculated that CAH may predispose to adrenal tumor formation. In support of this hypothesis, nodularity and adenomatous changes have been reported in adrenals of all types of CAH including the classic and nonclassic forms of CAH due to 21-OHD and at a young age [13–16]. Other stimuli, such as androgens, cytokines, and unknown paracrine factors may also contribute to adrenal tumor formation (Fig. 11.2).

The majority of published reports of adrenal tumors in CAH are single case reports; however, increased prevalence of adrenal tumors has been

found in a few case series studies, with tumor formation present in 11–82% of CAH patients (Table 11.1) [5, 6, 17].

Falke et al. examined adrenal gland characteristics by computed tomography (CT) in untreated patients with CAH ($n=6$, age 30–73 years, 4 patients with 21-OHD, 2 females with 11- β [beta] hydroxylase) and glucocorticoid treated patients with CAH ($n=7$, age/sex not reported). Adrenal hypertrophy was observed in 77% of the cohort and adrenocortical tumors were found in 23% [18]. Similarly, Harinaryana et al. examined CT characteristics in 6 children with untreated CAH (age 2–18 years, 5 with 21-OHD, and one female with 3- β [beta] hydroxysteroid dehydrogenase deficiency CAH) and found adrenal hypertrophy in 83% and adrenocortical tumors in 33% [14]. The finding of increased frequency of adrenal tumors in untreated CAH patients supports the role of chronic ACTH excess as a key adrenal trophic factor.

Jaresch et al. examined the frequency of adrenal tumors in 22 patients with CAH (age 12–60 years, 20 patients with 21-OHD, 1 female 11- β [beta] hydroxylase and one female with 3- β [beta] hydroxysteroid dehydrogenase deficiency CAH) using CT, and found 91% with adrenal hypertrophy and 82% with adrenocortical tumors [17]. Adrenal hypertrophy was based on the maximal cross-section of both adrenals. The extent of the hypertrophy correlated with the severity of the CAH phenotype. Bilateral tumors were observed in two patients and tumor size ranged from 0.5 to 5 cm. They found no correlation between tumor size and adrenal steroid levels; however, age and age at diagnosis were positively correlated with overall adrenal size supporting the concept that the length of time exposed to an altered hormonal milieu and developmental abnormalities play a role in promoting adrenal hypertrophy [17].

Giacaglia et al. in their prospective cohort study evaluated adrenal morphology by CT or magnetic resonance imaging (MRI) in 26 CAH patients. Patients were classified into four categories—untreated, poor, regular, and good control based on hormonal evaluation and history of glucocorticoid use. Adrenocortical nodules were found in 23% of

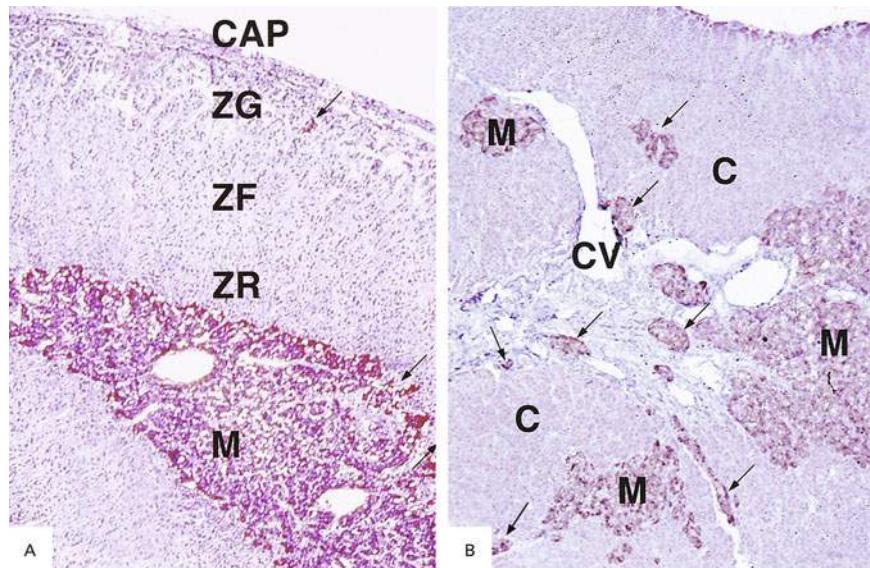


Fig. 11.1 Immunostaining of the adrenal gland. (a) In an unaffected adult, the adrenal shows well-defined zones and an inner medulla. (b) The adrenal of a female patient with classic salt-wasting congenital adrenal hyperplasia shows hyperplasia, poorly defined zones, and intermin-

gling of the inner medulla (magnification 40 \times). *CAP* capsule, *ZG* zona glomerulosa, *ZF* zona fasciculata, *ZR* zona reticularis, *M* medulla, *C* cortex and *CV* central vein. (From Merke et al [12], with permission)

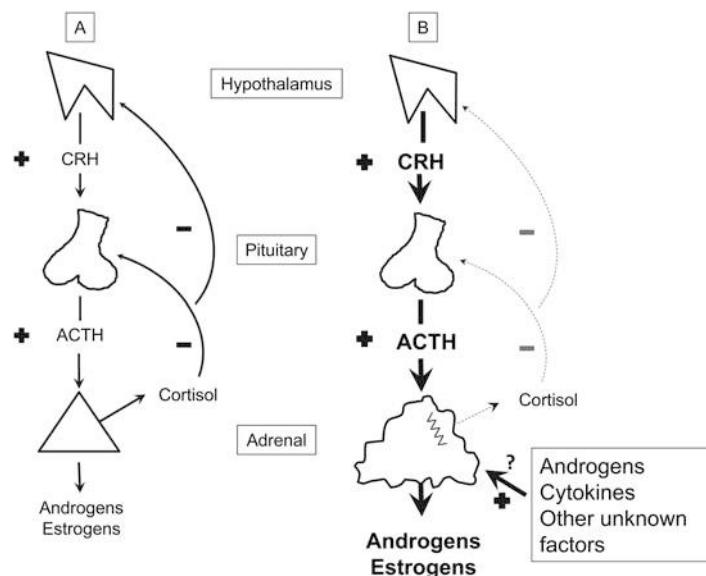


Fig. 11.2 Hypothalamic-pituitary-adrenal (HPA) axis. (a) In the normal physiologic HPA axis, CRH secreted from the hypothalamus stimulates secretion of ACTH from the anterior pituitary, which in turn stimulates cortisol synthesis in the adrenal cortex. Cortisol in turn exerts negative feedback at the levels of the hypothalamus and pituitary. (b) The HPA axis is altered in congenital adrenal hyperplasia. Lack of negative feedback of cortisol leads to hyperse-

cretion of CRH and ACTH. This continued uninhibited stimulation of the adrenal cortex leads to adrenocortical hypertrophy, overproduction of adrenal androgens, and contributes to tumor formation. Androgens, cytokines, and other unknown factors may also play a role in adrenal growth and tumor formation. *HPA* hypothalamic-pituitary-adrenal axis, *CRH* corticotrophin-releasing hormone, *ACTH* adrenocorticotropic hormone

Table 11.1 Adrenal imaging studies of patients with congenital adrenal hyperplasia

Reference	Sample size/age/sex	CAH type	Imaging modality	Adrenal morphology	Comments
Falke et al. [18]	n=13 Age: adults Sex: not reported	21-OH deficiency: 11 11 β -OH deficiency: 2	CT	Hypertrophy: 10 (77%) Adrenocortical tumors: 3 (23%) Nodule size: 1.5–5.5 cm	CAH diagnosis: hormonal evaluation
Harinayana et al. [14]	n=6 Age range 2 -18 years Sex: 4 F/2 M	21-OH deficiency: 5 3 β -HSD deficiency: 1	CT	Hypertrophy: 5 (83%) Adrenocortical tumors: 2 (33%) Nodule size: 1 cm	CAH diagnosis: hormonal evaluation
Jaresch et al. [17]	n=22 Age (mean \pm SD): 30.2 \pm 13.9 years (range 12–60) Sex: 17 F/5 M	21-OH deficiency: 20 (12 SV, 8 NC) 11 β -OH deficiency: 1 3 β -HSD deficiency: 1	CT	Hypertrophy: 20 (91%) Adrenocortical tumors: 18 (82%) Nodule size: 0.5–5 cm Bilateral tumors: 2	CAH diagnosis: hormonal evaluation. Tumor defined as a nodule >0.5 cm in diameter. No correlation between tumor size and adrenal steroid levels, and no differences in the frequency of tumors by age, or onset of treatment
Giacaglia et al. [19]	n=26 Age (mean \pm SD): 17 \pm 14.3 years (range 2.3–63) Sex: 21 F/5 M	21-OH deficiency: 26 (4 SW, 14 SV, 8 NC)	CT or MRI	Adrenocortical tumors: 6 (23%) Nodule size: 0.9–2.8 cm	CAH diagnosis: hormonal evaluation
Reisch et al. [5]	n=26 cases and n=26 age-matched controls Age (median): 33 years (range 18–48) Sex: 26 M	21-OH deficiency: 26 (15 SW, 11 SV)	MRI	Adrenal hypertrophy: 11 (42%) Adrenal nodularity: 19 (73 %), of whom 15 (57%) had nodule size >0.5 cm Nodule size: 0.6–3.7 cm	CAH diagnosis: genetic analysis Nodules defined as being present in at least one plane and evident in multiple sequences with a diameter of at least 0.5 cm. Nodule size correlated positively with hormonal control (17-OHP and androstenedione)
Nermoen et al. [6]	n=62 Age (median): 39 years (range 19–72) Sex: 39 F/23 M	21-OH deficiency: 62 (32 SW, 30 SV)	CT	Adrenal hypertrophy: 36 (58%) Adrenal tumors: 7 (11%) <ul style="list-style-type: none">• Myelolipoma 4• Pheochromocytoma 1 Nodule size: 1.2–16.5 cm Bilateral tumors: 2	CAH diagnosis: genetic analysis

CAH congenital adrenal hyperplasia, CT computed tomography, MRI magnetic resonance imaging, n number, F female, M male, 21-OH 21-hydroxylase, 11 β -OH 11 β [beta] hydroxylase deficiency, 3 β -HSD 3 β [beta]hydroxysteroid dehydrogenase, SW salt wasting, SV simple virilizing, NC nonclassic, 17-OHP 17-hydroxyprogesterone

patients (5 simple virilizing, 1 nonclassic) and tumor nodule size ranged from 0.9 to 2.8 cm. Nodules were identified only in patients with poor CAH control or in patients who were not on glucocorticoid therapy [19]. These findings are in line with studies by Falke et al. and Harinayana et al. and support the role of ACTH in adrenal hyperplasia and tumor formation. Adequate glucocorticoid therapy was initiated and maintained in the six patients with adrenal nodules. All had good hormonal control at the time of repeat imaging performed a year later which demonstrated considerable decrease in size in 3 patients (size range: pretreatment: 1.2–2.8 cm; posttreatment: 1–2.2 cm) and disappearance of tumors in three patients (size range: pretreatment: 0.9–2 cm). The changes in tumor size posttreatment emphasize the importance of adequate glucocorticoid therapy in the treatment of these ACTH-dependent adrenal tumors in CAH.

In a cross-sectional study by Reisch et al. adrenal morphology was evaluated by MRI in 26 adult males with CAH and 26 age-matched controls [5]. Adrenal hypertrophy was present in almost half of the patients with CAH and the majority had adrenal nodularity. As expected, adrenal nodularity was more common in CAH patients than controls (73 vs. 31%). In the patients with CAH, nodule size correlated positively with hormonal control (17-OHP and androstenedione). Similarly, in a cross-sectional population-based study in Norway, Nermoen et al. assessed CAH adult patients using adrenal CT. Adrenal hypertrophy was found in 58% of the CAH patients ($n=62$, age 18–75 years) and found positive correlations with ACTH and 17-OHP levels. They observed a higher frequency of adrenal tumors in CAH patients compared to the general population (11 vs. 2%) [6, 20]. Seven patients had one or more adrenal tumors with a diameter size ranging from 1.2 to 16.5 cm. Patients with adrenal tumors had significantly higher ACTH levels than those without tumors, but no differences were observed in 17-OHP levels.

Although large cohort imaging studies of adrenal morphology in patients with CAH are lacking, there is clear evidence that patients with

CAH commonly have adrenal hypertrophy and adrenal tumor formation. In addition, this risk appears to increase with age and lack of hormonal control.

Adrenal Myelolipoma

An increased frequency of adrenal myelolipomas has been reported in patients with CAH [6]. Adrenal myelolipoma is the most common fat-containing tumor of the adrenal gland [21]. In the general population, these benign nonfunctioning tumors are identified incidentally on imaging studies or during autopsy. Autopsy studies report an incidence of 0.08–0.4%, with an estimated incidence of 6% in classic CAH based on a study of 62 patients with 21-OHD (see Table 11.1) [6, 22, 23].

Adrenal myelolipomas are estimated to account for approximately 1.5–15% of all primary adrenal masses and occur equally in both men and women [6, 24–29]. Adrenal myelolipomas are rare, hormonally inactive benign tumors, and are composed of mature adipose tissue and hematopoietic (erythroid, myeloid, lymphoid) components of varying proportions [23, 30]. They are typically unilateral, but multiple and bilateral myelolipomas have been reported [31, 32]. In cases of unilateral lesions, there appears to be a predominance on the left side and in individuals with bilateral myelolipomas, left-sided masses are generally larger compared to the right [32]. The location of the abdominal organs possibly accounts for this discrepancy, with right-sided growth restricted by the liver [32, 33].

Although adrenal myelolipomas are benign tumors and are usually identified serendipitously, large adrenal myelolipomas often come to clinical attention secondary to symptoms from pressure on the adjacent organs. Several case reports highlight the clinical presentation of giant adrenal myelolipomas which can present with chronic abdominal/flank pain, emesis, constipation from mechanical compression on the bowels, urinary tract infections from compression on the kidneys/ureters or acute abdominal pain due to spontaneous rupture, necrosis, and hemorrhage [32, 34–38]. Size does not

always correlate with the severity of symptoms [39, 40]. Approximately, 70% of reported cases of giant (defined as >6 cm) adrenal myelolipomas in CAH have been diagnosed based on symptomatology, most commonly abdominal pain and increasing abdominal girth, but symptoms also include dyspnea, back pain, nausea, and vomiting (Table 11.2). The largest resected myelolipoma in a patient with CAH was reported by Allison et al. [41]. The patient presented with increasing abdominal girth and a 40 pound weight loss over a 2-year period. The mass weighed 9.65 kg and measured 43×33×16 cm [41].

Although myelolipomas are commonly reported in CAH due to 21-OHD, these benign tumors are reported in all CAH types [42–44]. Adrenal myelolipomas in patients with CAH are especially described in patients with poor compliance or in undiagnosed cases, and multiple or bilateral tumors are commonly found (see Table 11.2) [45–53]. Myelolipomas are also seen in other endocrine disorders with ACTH excess, such as Cushing's disease, and Nelson syndrome [54, 55]. The association of these tumors in disease states with elevated ACTH suggests that chronic excess of ACTH plays a role in tumor formation.

The exact pathogenesis of myelolipoma remains uncertain and several theories exist. A widely accepted hypothesis is that these tumors arise from metaplasia of undifferentiated stromal cell of the zona fasciculata of the adrenal cortex in response to stimuli such as infection, stress, tissue necrosis, chronic ACTH, and/or androgen levels [23, 35, 37, 39, 56, 57]. Other less favored theories include the development of myelolipoma from intra-adrenal embryonic rests of bone marrow and adrenal embolism of bone marrow cells, chromosomal inactivation, or translocation [58, 59]. The higher preponderance of these fat-containing tumors in adult life supports the hypothesis that long-standing stimulation plays a role [23, 35, 56]. To our knowledge there are no case reports of adrenal myelolipomas in children with CAH. The youngest patient with CAH with a documented myelolipoma seen at our center, the National Institutes of Health (NIH) Clinical Center, was 19 years old (Fig. 11.3).

Histopathology

Cross section of adrenal myelolipoma shows yellowish areas of fat and reddish brown areas of myeloid elements (Fig. 11.4a) [35, 60]. A well-defined pseudocapsule can be seen, sometimes with spotty to diffuse calcifications [35, 39, 53]. The histopathology of myelolipoma shows mature adipose tissue and varied amounts of myeloid (hematopoietic) tissue. Bone, along with remnants of normal to hyperplastic adrenal cortex, may also be observed (Fig. 11.4b) [61, 62].

Radiological Characteristics

Imaging features of myelolipomas vary based on their histologic composition. The fat content of these tumors has key features which help in the diagnostic workup of these benign lesions. On ultrasonography, myelolipomas with more myeloid elements will appear hypoechoic and lesions predominantly containing fat appear hyperechoic [63, 64]. CT is the most sensitive imaging modality and CT features vary from well-delineated, heterogeneous masses with mixed areas of negative attenuation (less than -30 HU, often less than -100 HU) due to the fat content and soft tissue attenuation (20–50 HU) (Fig. 11.5) [53, 64, 65]. Areas of increased density noted on CT reflect the uptake by myeloid components of these tumors [66]. Small, punctate calcifications can be seen on CT [64, 67].

Heterogeneous appearance of these tumors on CT can raise the suspicion for malignant lesions such as adrenocortical cancer or retroperitoneal liposarcoma [53]. Unlike other fat-containing benign tumors, myelolipomas can demonstrate increased uptake with ¹⁸F-fluorodeoxyglucose positron emission tomography-CT imaging corresponding to the adenomatous and hematopoietic components [38, 68].

On MRI, the fat component of a myelolipoma usually has a high intensity signal and the myeloid component typically has low intensity on T1-weighted images (nonfat suppressed). Variable features are seen on T2-weighted MRI images ranging anywhere from moderate to intermediate

Table 11.2 Case reports of giant (>6 cm) adrenal myelolipomas in patients with congenital adrenal hyperplasia^a

No.	Reference	Age/sex	CAH type/phenotype/genotype	Location/size of lesion [right (R), left (L)]	Imaging modality	Clinical presentation
1	Boudreaux et al. [35]	57/M	21-OHD ^{b,c} SV	L: 34×24×10.5 cm ^d	Intravenous pyelogram, aortogram CT	Dyspnea, lower thoracic pain, increases in abdominal girth. CAH diagnosis made at the time of presentation
2	Condom et al. [43]	50/F	17α-OHD ^{b,c}	L: 15×12×9 cm	CT	Hypertension, acute renal failure. CAH diagnosis made at the time of presentation
3	Oliva et al. [48]	34/F	21-OHD ^{b,c} SV	L: 13×6×5 cm	CT & MRI	L flank pain, hematuria, and fever. Off glucocorticoid therapy for 20 years
4	Ravichandran et al. [36]	58/M	21-OHD ^{b,c} SV XX male	R: 7×10 cm (L: size not reported)	Intravenous pyelogram & CT	Recurrent urinary tract infections. CAH diagnosis made at the time of presentation
5	Parenteau et al. [49]	28/M	21-OHD ^c SW	L: 12.5×6×9 cm	CT & MRI	Abdominal pain, fatigue, asthenia. History of noncompliance and was off glucocorticoid therapy for 4 years
6	Nagai et al. [44]	45/F	17α-OHD ^{b,c} XY female	L: 10×6 cm	MRI	Hypertension and hypokalemia. CAH diagnosis made at the time of presentation
7	Allison et al. [41]	43/M	21-OHD ^c SV XX male	L: 43×33×16 cm R: 24×19×12 cm	CT	Increase in abdominal girth despite 40 pound weight loss in 2 years. Receiving hydrocortisone therapy
8	Patocs et al. [42]	37/F	17α -OHD ^b Genotype: p.R440C	L: 7 cm (R: 2 cm)	CT	Hypertension and hypokalemia. CAH diagnosis made at the time of presentation
9	Kalidindi et al. [53]	42/M	21-OHD ^c SW	L: 25×25×14 cm R: 23×17×15 cm	CT	Evaluation of ruptured quadriceps tendon and L sided abdominal mass noted on exam. Off glucocorticoid therapy for 11 months
10	Mathew et al. [50]	62/F	21-OHD ^{b,c} SV	L: 11×12×10 cm R: 6×4×5 cm	CT	Fatigue, breathlessness with a febrile illness. CAH diagnosis made at the time of presentation
11	Sakaki et al. [45]	69/F	21-OHD ^b SV Genotype: p.Q318X/unknown ^e	L: 8×5 cm (R: 4×3 cm)	CT	Nausea, vomiting. CAH diagnosis made at the time of presentation
12	Hagiwara et al. [51]	43/F	21-OHD SV Genotype: I2G/p.I172N	L: 15×15×10 cm (R: size not reported)	CT	Incidental finding on upper GI series. Off glucocorticoid therapy for >20 years
13	John et al. [16]	23/M	11β-OHD ^{b,c}	L: 10×10×8 cm R: 6.5×3.7×2.8 cm	CT	Hypertension and L adrenal mass. CAH diagnosis made at the time of presentation

(continued)

Table 11.2 (continued)

No.	Reference	Age/sex	CAH type/pheno/ genotype	Location/size of lesion [right (R), left (L)]	Imaging modality	Clinical presentation
14	Mermejo et al. [52]	57/M	21-OHD SV Genotype: p.Q318X/I2G	L: 15×10 cm	CT & MRI	Abdominal pain. CAH diagnosis made at the time of presentation
15	Peppa et al. [46]	61/F	21-OHD SV Genotype: p.I172N/I2G	L: 9×6 cm ^d	MRI	Incidental finding. History of on and off on glucocorticoid therapy
16	German-Mena et al. [32]	45/M	21-OHD ^e SV	L: 24.4×19.0×9.5 cm R: 6×5.5×6 cm	CT	Increasing abdominal girth and abdominal pain. Off glucocorticoid therapy for 35 years
17	Ioannidis et al. [47]	34/F	21-OHD ^e SV	L: 24×14×10 cm R: 16×11×8 cm	CT & MRI	Abdominal pain and vomiting. Off glucocorticoid therapy – unknown duration
18	McGeoch et al. [7]	34/M	21-OHD SV	L: 23×11×19 cm R: 15×13×6.8 cm	CT	Abdominal swelling and discomfort. Off glucocorticoid therapy for 1 year
19	Almeida et al. [37]	Case 1: 35/F Case 2: 52/F	Case 1: 21-OHD SV Genotype: compound heterozygous [p.E35IV and exon 6 cluster (p.I236N, p.V237E, and p.M239K)] Case 2: 21-OHD SV Genotype: I2G/p.I172N	Case 1: L: 14×14×10 cm R: 8.9×8.3×8 cm Case 2: L: 16×13×9 cm R: 6.9×5.3×4.3 cm	CT	Case 1: abdominal pain, off glucocorticoid therapy for 15 years Case 2: abdominal pain CAH diagnosis made at the time of presentation
20	Al-Bahri et al. [33]	39/M	21-OHD ^e SV	L: 30×25×20 cm R: 25×20×13 cm	CT	Abdominal distension, fatigue, decreased libido, and easy bruising. Off glucocorticoid therapy since adolescence
21	Kale et al. [27]	51/M	21-OHD ^e SW	L: 34×20×13 cm R: 20 cm	MRI	Chronic lower back pain and new onset lower extremity paresthesias. Patient receiving prednisone therapy

M male, *F* female, *21-OHD* 21-hydroxylase deficiency, *11β-OHD* 11β-hydroxylase deficiency, *17α-OHD* 17α-hydroxylase deficiency, *SW* salt-wasting, *CT* computed tomography, *MRI* magnetic resonance imaging, *cm* centimeter

^aArticles in English language are included
^bDiagnosis based on hormone evaluation
^cGenotype not provided

^dMass was resected en bloc and included kidney and retroperitoneal soft tissue [35]
^eReported variants of unknown significance

intensity mostly due to a combination of fatty areas mixed with myeloid tissue. The demonstration of fat is best achieved with fat suppression imaging techniques that will show loss of signal intensity with the fatty areas of the lesion and increased intensity with myeloid components [69]. This technique may assist in discriminating myelo-

lipoma from adenoma [6, 64, 70]. Hemorrhage within the myelolipoma has variable intensity depending on the stage of the hemorrhage.

Functional Tumors

Pheochromocytoma

To our knowledge, there is one report of a pheochromocytoma in a patient with CAH. This patient was identified incidentally as part of a cross-sectional study ($n=62$) evaluating the radiological characteristics of the adrenals of patients with CAH [6]. Similarly, we have diagnosed one patient with pheochromocytoma in our cohort of 128 adults with CAH (unpublished). Our patient was incidentally found to have an adrenal mass (Fig. 11.6). Although he had a history of intermittent elevated blood pressure readings, he was otherwise asymptomatic. Biochemical workup was diagnostic for a pheochromocytoma with markedly elevated 24-h total urinary metanephrenes at 1573 mcg/24 h (normal 200–614), and normetanephrenes at 1,519 mcg/24 h (normal 111–419), and elevated plasma fractioned normetanephrenes at 516 pg/mL (normal 18–112) and norepinephrine at 1420 pg/mL (normal 80–498). Our patient

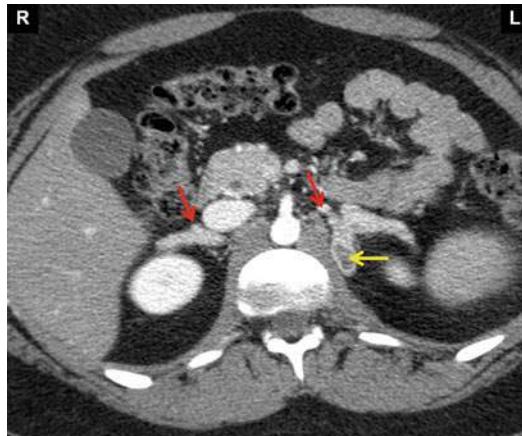


Fig. 11.3 Adrenal computerized tomography image of a 19-year-old male with classic simple-virilizing congenital adrenal hyperplasia shows moderate to marked diffuse enlargement and nodularity (red arrows) of both adrenal glands. The lesion in the left adrenal gland has a fatty radiodensity (yellow arrow) consistent with the diagnosis of myelolipoma. R right, L left

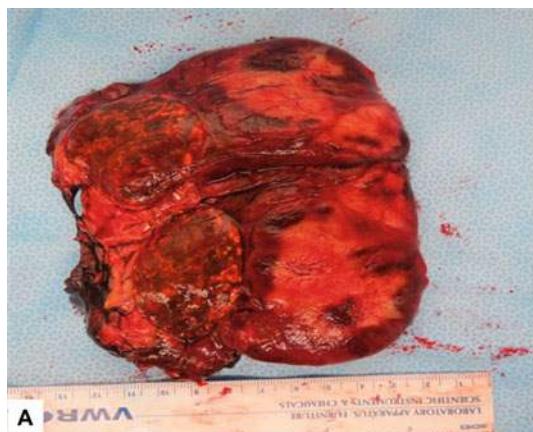
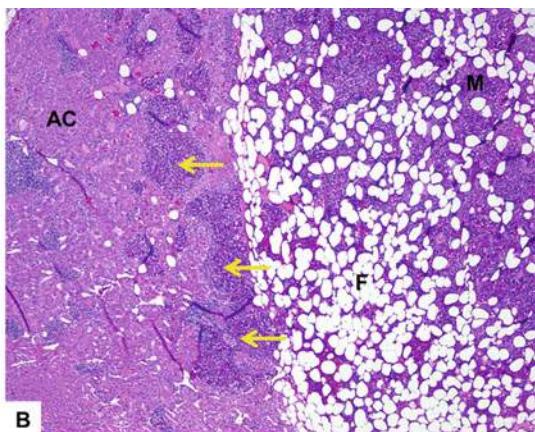


Fig. 11.4 Adrenal myelolipoma from a 29-year-old female with classic simple-virilizing congenital adrenal hyperplasia. (a) Gross specimen of the left adrenal myelolipoma measures $11.5 \times 8.3 \times 4$ cm and weighs 187 g. The bisected specimen has a heterogeneous maroon and tan soft cut surface with golden orange flecks throughout. (b)



Histopathology shows hyperplastic adrenocortical cells, mature adipose tissue, and myeloid (hematopoietic) precursor cells. Islands of myeloid cells are found within the adrenal cortex (yellow arrows). Magnification 40 \times , stain hematoxylin & eosin. AC adrenal cortex, F fat component of myelolipoma, M myeloid component of myelolipoma

underwent curative laparoscopic resection of the mass. Although pheochromocytoma is rare in individuals with CAH, pheochromocytomas are reported in up to 11.5% (0.4–11.5%) of patients with adrenal incidentaloma, depending on the cohort and duration of follow up [71–76].



Fig. 11.5 Adrenal computerized tomography image of a 29-year-old female with classic simple-virilizing congenital adrenal hyperplasia shows hyperplasia of right adrenal gland with small nodules (white arrow) and a lobular mass of 5×3 cm involving the left adrenal gland. The upper mass (yellow arrow) (5 cm) contains mostly fatty tissue [-64 Hounsfield Units (HU)] and the lower portion (red arrow) is of soft tissue density (49 HU). R right, L left

Therefore, occasional cases might be expected in patients with CAH. This requires further study.

Adrenocortical Carcinoma

Adrenocortical carcinoma is rare in the general population and carries a poor prognosis [77, 78]. Adrenocortical carcinoma has been reported in two adults and two children with classic CAH, and three of these patients were untreated and previously not diagnosed with CAH [79–82]. The etiology of the development of adrenocortical carcinoma in the patient with CAH is unknown, but chronic stimulation of the adrenal cortex by ACTH and overproduction of adrenal steroids coexist in reported cases. Thus, the development of adrenocortical carcinoma in a diagnosed patient receiving proper treatment would be extremely rare, especially given the rarity of adrenocortical carcinoma in general.

The histopathologic distinction between adenoma and carcinoma is challenging in adrenocortical tumors and is especially challenging in the CAH patient. Histological and immunohistochemical analyses revealed massive lymphocytic infiltration, increased mitotic rate, atypical mitotic

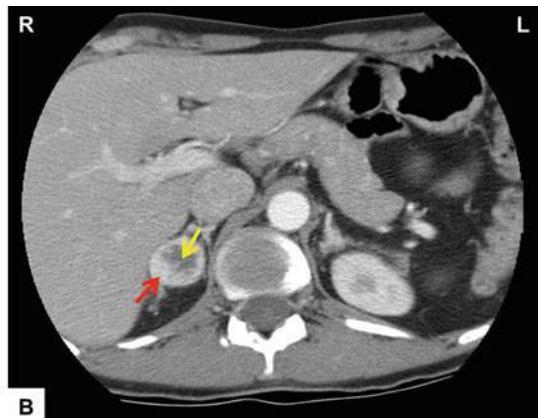


Fig. 11.6 Adrenal computed tomography images of a 31-year-old male with classic salt-wasting congenital adrenal hyperplasia who was incidentally found to have an adrenal mass. (a) The lesion appears heterogeneous, with ill-defined borders and a precontrast density of 26 Hounsfield Units (HU) in the center, and 35 HU at the periphery of the lesion (white arrow). (b) The postcontrast

image shows a heterogeneous mass with an irregular border, thick walls (red arrow), and a necrotic center (yellow arrow), and a density of 39 HU in the center, and 174 HU at the periphery of the lesion. The patient underwent curative surgery following a definitive biochemical and imaging workup for pheochromocytoma. R right, L left

figures, and a high nuclear grade suggestive of adrenocortical carcinoma in a patient with classic salt-wasting CAH and large nodular adrenals, but she did not have adrenocortical carcinoma [83]. She did not have metastases, bilateral adrenalectomy was performed and there is no evidence of recurrence 20 years later. Hayashi et al. reported a 68-year-old XX male presenting with a virilizing adrenocortical mass and subsequently diagnosed with CAH due to 21-OHD [84]. Although the tumor was diagnosed as adrenocortical carcinoma based on satisfying four Weiss criteria for malignancy (sinusoidal invasion, venous invasion, cytoplasm, and capsular invasion), surgery appears to have been curative, thus the diagnosis of malignancy is uncertain. Similarly, Bhatia et al. and Chevalier et al. describe cases of untreated classic CAH with adrenal masses suggestive of carcinoma but malignancy was not proven [15, 85]. Overall, the development of adrenocortical carcinoma in a patient with CAH would be extremely rare, but suspicious histologic changes of adrenal cells may not be that uncommon in the untreated patient. This is an area that requires further investigation.

Management of the Adrenal Mass

Adrenal imaging is not recommended in the routine care of CAH patients. The Endocrine Society Clinical Practice Guideline for CAH recommends adrenal imaging be reserved for CAH patients who have an atypical clinical or biochemical course [3]. Patients presenting with signs and symptoms of virilization despite proper treatment, patients with abdominal pain or undiagnosed patients, may undergo adrenal imaging (CT or MRI). Adrenal nodules may also be detected incidentally if a patient with CAH undergoes imaging studies for other indications.

Most adrenal nodules in CAH patients are benign nonfunctioning lesions and are often bilateral. The approach to the patient with CAH patient with an adrenal mass should be based on patient symptomatology and the radiological characteristics of the nodule (Fig. 11.7) [86]. Biochemical evaluation is especially recommended in the evaluation of adrenal masses with specific characteristics on imaging studies indicative of nonadenomatous lesions (Fig. 11.8). This includes nodules which are nonhomogenous, have irregu-

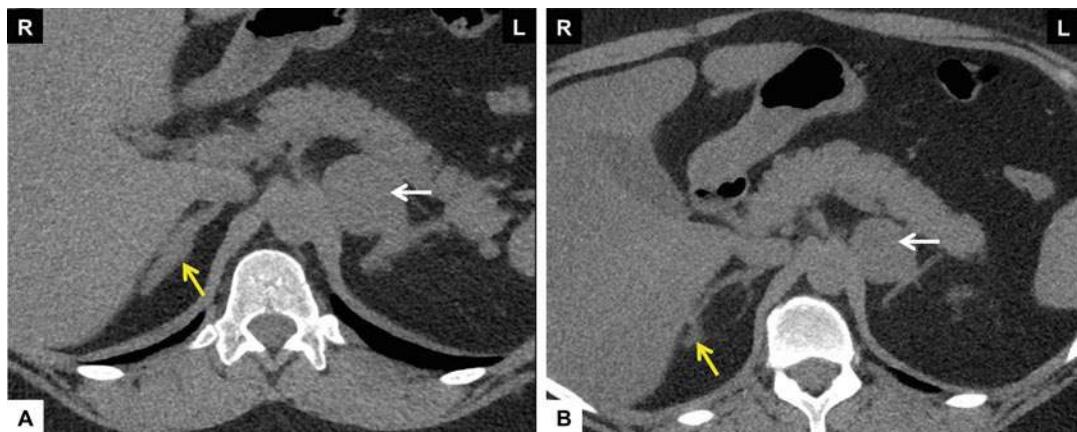


Fig. 11.7 Adrenal computerized tomography (CT) scan images of a 38-year-old female with classic salt-wasting congenital adrenal hyperplasia with a long-standing history of hyperandrogenism. **(a)** Diffuse bilateral adrenal gland hypertrophy and nodularity are seen (yellow arrow), with a dominant $4.2 \times 3.4 \times 4.1$ cm mass arising from the midportion of left adrenal gland (white arrow). There is no evidence of macroscopic fat within this lesion to suggest a myelolipoma. Precontrast CT density is 29

Hounsfield Units (HU), also not typical for an adenoma **(b)** Repeat adrenal CT 8 months postimproved glucocorticoid therapy demonstrates minimal nodularity (yellow arrow) in the right adrenal gland and a decrease in the size of the left adrenal gland mass to $2.9 \times 3.6 \times 3.6$ cm. Precontrast density measures 29 HU, and postcontrast washout is 54%. The washout characteristics in combination with a decrease in the size of the adrenal nodule suggest a benign adenoma. *R* right, *L* left

lar borders, precontrast density >10 HU, and contrast washout <50% on CT scan [86].

As current literature is limited on the natural history of adrenal nodules in CAH, follow up of incidentally detected masses in CAH patients should mostly follow current guidelines in the management of adrenal incidentalomas, with the caveat that the patient with CAH is at increased risk for benign adrenal tumor formation [87].

Given the higher risk of malignancy in lesions >6 cm, the risk of symptomatology due to compression of neighboring organs and the risk of tumor infarction, consideration for adrenalectomy and increased monitoring is recommended for large tumors [88].

In the patient with CAH with an adrenal mass, improved management of CAH is indicated and follow-up radiological evaluation should be inter-

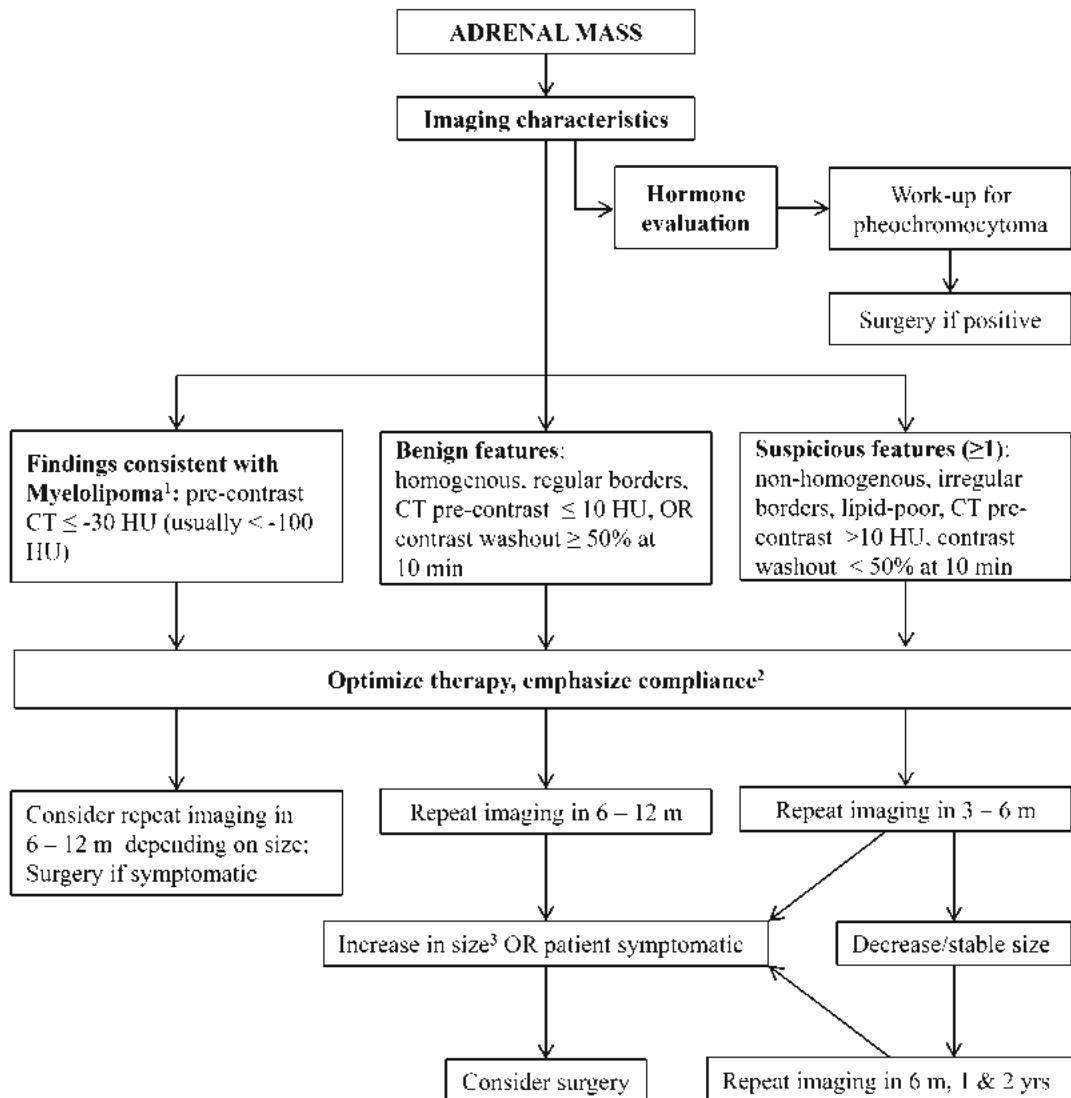


Fig. 11.8 Suggested evaluation of an adrenal mass in a patient with congenital adrenal hyperplasia. *CT* computed tomography, *HU* Hounsfield units, *m* months, *yrs* years.
¹Myelolipomas can appear as heterogeneous masses and show areas of increased density on CT, reflecting the

increased uptake by myeloid components of these benign nonfunctioning tumors. ²In addition to optimizing therapy, consider a short course of dexamethasone, and, in select cases, a pituitary MRI. ³Growth of 0.5–1 cm/year

preted with consideration of the degree of CAH control (see Fig. 11.7). An example of this is a 38-year-old female with classic salt-wasting CAH with long-standing history of hyperandrogenism and poor control seen at the NIH Clinical Center. An adrenal CT revealed bilateral adrenal gland nodularity, with a dominant $4.2 \times 3.4 \times 4.1$ cm mass arising from the midportion of left adrenal gland. The patient was initiated on higher dose glucocorticoid replacement given in a more physiologic manner. Repeat adrenal CT 8 months postimproved therapy demonstrated a decrease in nodularity and a decrease in the left adrenal nodule $2.9 \times 3.6 \times 3.6$ cm. Although precontrast CT density was suspicious (precontrast measured 29 HU), washout characteristics were suggestive of a benign adenoma (see Fig. 11.7).

Theoretically, long-standing adrenal nodules may have the potential to function autonomously and not under the control of the HPA. In our clinical practice, we use the dexamethasone suppression test to evaluate functionality of nodules found in the poorly controlled patient. If an overnight dexamethasone test does not suppress 17-OHP then an MRI of the pituitary should be considered to rule out a pituitary adenoma. Corticotropinoma in CAH is rare, but can occur [89]. In addition, a short course of dexamethasone 0.5–1 mg for 7–10 days, followed by an increase in daily glucocorticoid dosage may achieve improved hormonal control in patients with significantly elevated adrenal androgens. Adrenal steroid levels should be repeated in 4–6 weeks and repeat imaging performed in 6 months to monitor tumor growth.

The natural course of myelolipomas in CAH is unknown. Follow-up studies of adrenal myelolipoma support a conservative approach in the management of small adrenal myelolipomas as they tend to remain stable. Routine follow up may not be necessary unless symptoms arise as these hormonally inactive tumors exhibit variable growth and bleed infrequently [39]. However, large-sized tumors need surgical intervention if patients are symptomatic [39, 90]. Large adrenal myelolipomas occur in the setting of poor compliance or undertreatment in CAH patients.

Role of CYP21A2 in Adrenal Tumor Formation

In general, most adrenal nodules are identified as incidentalomas and more than two-thirds are benign nonfunctioning adenomas [25, 26, 75]. The role of CYP21A2 in tumor formation has been investigated in several studies because of the known development of adrenal tumors in CAH patients. Moreover, in a study of 20 CAH carriers, 45 % were found to have adrenal nodules ranging from 0.5 to 5 cm in size suggesting that asymptomatic carriers may be at risk for adrenal tumor formation [17]. Supporting this notion, in multiple studies, exaggerated increases in 17-OHP levels following ACTH stimulation have been found in 30–70 % of patients undergoing an endocrine evaluation for an adrenal incidentaloma [91–93]. However, caution should be used in interpreting these results because tumors may be associated with altered steroidogenesis leading to high 17-OHP without the presence of CYP21A2 mutations [92, 94, 95].

CYP21A2 genetic studies have been performed in studies of adrenal incidentalomas. Baumgartner-Parzer et al. found CYP21A2 carrier status in 16 % (8/50) of individuals with non-functioning adrenal adenomas [96]. Patocs et al. identified CYP21A2 mutations (one homozygous and three heterozygous) in 21 % (4/19) of patients with bilateral adrenal incidentalomas and in 16 % of patients with unilateral masses [97]. Two population-based studies compared CYP21A2 mutation frequency between patients with adrenal incidentaloma and healthy controls [98, 99]. Doleschall et al. found no difference in CYP21A2 carrier state between adrenal incidentaloma patients and healthy controls (9.6 vs. 10.3 %), while Kiedrowicz et al. found a higher CYP21A2 carrier rate in patients with adrenal incidentalomas compared to controls (8 vs. 0 %). CYP21A2 mutation status has not consistently correlated with hormonal testing and tumor size [94]. A recent meta-analysis of thirty-six studies of CAH prevalence in adrenal incidentaloma patients found that 5.9 % (58/990) of patients screened positive for CAH by biochemical analysis. But genetic analysis confirmed CAH in only 0.8 % of

all adrenal incidentaloma cases [13]. A lack of detailed clinical and genetic reporting in the majority of the studies limited this meta-analysis, but the results overall do not support routine screening for *CYP21A2* mutations in patients with adrenal incidentaloma.

Although CAH is an uncommon diagnosis in the workup of adrenal incidentalomas, CAH screening should be considered in select cases, especially in the presence of bilateral masses [29, 86, 100]. It remains possible that undiagnosed CAH patients or CAH carriers might be overrepresented in patients with adrenal incidentalomas, and the presence of bilateral tumors increases the likelihood of CAH. In published studies of adrenal incidentaloma, approximately one-third of the patients biochemically diagnosed with CAH had bilateral masses and over one-half of the patients with genetically confirmed CAH had bilateral masses, which is higher than the overall prevalence of bilateral masses of 11–19% [13, 71, 75, 76, 101]. Given the lack of specificity and sensitivity of elevated 17-OHP in the patient with an adrenal incidentaloma, genetic testing is preferred over biochemical testing to rule out CAH, particularly if surgical intervention is under discussion [13]. Adrenalectomy without knowledge of a CAH diagnosis could result in an adrenal crisis in the postoperative period.

Conclusion

CAH, the most common genetic cause of primary adrenal insufficiency, is characterized by alterations in the HPA axis which predisposes patients to adrenal tumor formation. Benign adrenal adenoma and myelolipoma are the most common tumors found in patients with CAH and are often bilateral. Routine adrenal imaging is not recommended in the management of patients with CAH but, when adrenal masses are identified, consideration should be given to the types of tumors frequently found. In CAH, adrenocortical masses are most commonly reported in older patients, and in those with poor-disease control. Thus, first-line therapy is to optimize glucocorticoid treatment. This often results in tumor shrinkage emphasizing the need for preventative patient

education regarding compliance and also reflecting the need for improved therapies.

Given the overall increase in the use of imaging in medical practice and the common finding of adrenal incidentalomas, familiarity with tumor characteristics in routinely used imaging techniques will avoid unnecessary procedures and anxiety in patients. Though CAH is an uncommon diagnosis in the workup of adrenal incidentalomas, screening for CAH should be considered in select cases, especially in the presence of bilateral masses.

Future studies of tumor formation in CAH will expand our understanding of the etiology of adrenal tumor formation. New and improved therapies are being developed that aim to replace cortisol in a physiological manner, and a reduction in HPA stimulation with less ACTH release is expected with treatments that mimic cortisol circadian secretion [102]. Thus, the development of improved therapies may contribute to the future prevention of adrenocortical tumor formation in patients with CAH.

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Presentation

Adrenocortical carcinoma (ACC) is a rare malignancy, with incidence rates varying from 0.7 to 2.0 cases per million population each year [1–3]. Most ACCs occur sporadically, but they can also be part of various genetic syndromes such as Li–Fraumeni syndrome [4], Beckwith–Wiedemann syndrome [5], or Multiple Endocrine Neoplasia 1 [6]. ACCs can occur at any age, with an incidence peak before the age of 5 years and around the age of 40–50 years [7, 8]. Women are more often affected than men (1.5:1) [1] and ACCs are thought to be more prevalent on the left side of the body, although the mechanism or biological explanation behind this observation is still unknown. ACCs predominantly do not present with the most common symptoms of malignancy, such as night sweating, fever, and weight loss [9]. In contrast, approximately 40–60% of ACCs secrete sufficient adrenal steroids to present with clinical symptoms due to hormone excess [7, 8]. It is important that in these patients clinical features due to hormonal production are recognized

by physicians, so that appropriate imaging and clinical investigation can be performed. Table 12.1 summarizes the most prevalent clinical forms of ACC presentation. Patients with functional ACCs mostly present with features of Cushing’s syndrome (CS) (~55%) with easy bruising, facial plethora, proximal myopathy, muscle weakness, and purple striae [10]. In a subset of patients severe hypercortisolism develops rapidly over a few months and these patients may not have the typical CS phenotype. Rapidly progressing signs of cortisol excess are indicative for malignancy of the adrenal tumor. Very high cortisol levels can, due to saturation of the renal corticosteroid 11 β -hydroxysteroid dehydrogenase 2 (HSD11B2) system, also activate the mineralocorticoid receptor, leading to hypertension and severe hypokalemia [11]. Hypertension and hypokalemia can also be caused by high levels of aldosterone, although this is rare in ACC [12]. The second most frequent clinical presentation of functional ACCs is based on coexisting hypersecretion of cortisol and adrenal androgens (~25%). In these cases the typical CS features can be blunted. In approximately 10% of cases virilization (i.e., acne, oligomenorrhea, and hirsutism), due to isolated hyperandrogenism, is the clinical presentation. In male patients, symptoms of excess androgens can be difficult to recognize and are mostly diagnosed only when androgens are converted to estrogens in the peripheral tissues or by estrogen cosecretion by the ACC. Estrogen

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Table 12.1 Most prevalent forms of adrenocortical carcinoma presentation and corresponding laboratory evaluations to determine hormone excess

Clinical presentation	PR	Hormone	PR	Symptoms	Laboratory evaluations
Hormone excess	40–60 %	Cortisol	~55 %	Easy bruising, facial plethora, proximal myopathy, muscle weakness, purple striae, steroid-induced diabetes, psychiatric problems, hypertension, and hypokalemia	Basal ACTH and cortisol, 24 h urine collection, dexamethasone suppression test, midnight salivary cortisol
		Cortisol and androgens	~25 %	Cortisol- and androgen-mediated symptoms	Cortisol and androgen
		Androgens	~10 %	<i>Females:</i> acne, oligomenorrhea, hirsutism, clitoromegaly, androgenetic effluvium <i>Males:</i> aggressive behavior, decreased testicle volume	17-OH-progesterone, androstenedione, and DHEAS levels, free testosterone <i>Males and postmenopausal women:</i> 17 β -estradiol
		Estrogens	<5 %	<i>Males:</i> gynecomastia and testicular atrophy, loss of libido	Males and postmenopausal females: 17 β -Estradiol
		Aldosterone	<5 %	Hypertension and hypokalemia	Aldosterone:renin ratio, potassium
Tumor growth	30–40 %			Abdominal pain, fullness, a palpable mass, or early satiety	
Incidentally discovered	10–25 %			Adrenal mass visible on abdominal imaging	

In all patients 24 h urine catecholamine or plasma free metanephrenes should be collected to rule out a pheochromocytoma. PR prevalence, ACTH adrenocorticotrophic hormone, DHEAS dehydroepiandrosterone sulfate

excess, leading to feminization in men, occurs in less than 5 % of the cases [13]. An important consideration is that androgen or estrogen secreting adrenal tumors are suggestive of an ACC rather than an adrenocortical adenoma (ACA). Tumors without overt hormone excess often also produce adrenal steroid precursors. Although paraneoplastic syndromes are relatively uncommon in patients with ACC, tumor-associated hypoglycemia has been described repeatedly, probably due to release of insulin-like growth factor 2 (IGF2) [14, 15]. In some cases, tumor production of chemotactic chemokines can cause fever and leukocytosis [16].

Patients may also present with symptoms due to local or distant tumor growth, i.e. flank pain, abdominal discomfort, back pain, or abdominal fullness [7, 8].

About 10–25 % of the ACC cases are diagnosed incidentally on radiographic imaging, and

this percentage is still thought to be increasing due to the wide use of imaging studies in medicine. At the time of presentation, most ACCs are very large, measuring on average 10–13 cm, but can be localized [8, 17, 18]. The prevalence of incidentally discovered masses on CT examinations is 0.35–5.0 % [19].

Diagnosis

For decades, there has been debate regarding the optimal diagnostic strategy for patients with an incidentally discovered adrenal mass. Early and correct classification is relevant to establish the appropriate therapeutic strategy. In the last years, as a result of extensive research and international collaborations, existing diagnostic tools have been improved and new approaches have been proposed.

Hormonal Evaluation

It is recommended that a thorough hormonal evaluation be performed in all patients with (suspected) ACC, even in the apparently nonfunctional tumors [8]. First the adrenocortical origin of the tumor can be established in this way, and second other relevant diagnoses can be excluded. Measurement of 24 h urinary (nor)metanephries or plasma free (nor)metanephries should be performed to rule out a pheochromocytoma. There are several other reasons why hormonal evaluation needs to be performed prior to surgery: (1) It can give additional information regarding the risk of malignancy; (2) In case of glucocorticoid excess, postsurgical hydrocortisone replacement therapy is indicated; and (3) in order to use steroid hormones as a tumor marker for future surveillance and follow-up. Although part of the initial workup is guided by symptoms (see Table 12.1), all patients should be evaluated for hypercortisolism by first-line screenings test (24 h urinary cortisol excretion, 1 mg dexamethasone overnight test, or late night salivary cortisol levels). Dehydroepiandrosterone sulfate (DHEAS) and testosterone should also be measured in every patient. Measurement of blood pressure and serum potassium levels is required in case of a suspicion of cortisol or aldosterone production by the tumor.

Imaging

To support the decision on adrenalectomy, it is imperative to recognize adrenal tumors with malignant potential using imaging techniques. Initial evaluation of adrenal masses is usually performed with assessment of the radiological characteristics on (contrast-enhanced) computed tomography (CT) scan and/or magnetic resonance imaging (MRI) [20]. The most important predictor for malignancy is the size of a tumor, although size alone is not sufficient for an accurate discrimination between ACCs and ACAs [18]. For tumors ≥ 8 cm, the probability of malignancy is 47%, while this is only 10% for tumors ≥ 4 cm [18]. In a recent retrospective study, 2/20 patients with an

adrenal tumor smaller than 6 cm preceding the diagnosis of ACC had initial imaging characteristics of an ACA [21]. Other morphological features raising the suspicion of ACC include irregular margins, increased tumor heterogeneity (due to necrosis or hemorrhage), calcifications, central low attenuation, > 10 Hounsfield Units (HU), and extension into the inferior vena cava or renal veins [22]. Central low attenuation can be due to areas of necrosis, while calcifications are best detected as high attenuation foci. A recent analysis of the German ACC registry showed that a threshold of 13 HU instead of 10 HU was a more sensitive threshold for malignancy [23]. MRI can be used for vascular assessment and further evaluation of the adrenal mass, since ACCs appear to be isointense to hypointense relative to liver parenchyma on T1-weighted MRI and hyperintense on T2-weighted images [24]. Highly suggestive features of malignancy are a venous thrombus and lymphadenopathy. On enhanced CT, adenomas typically display rapid washout of contrast medium, whereas this is delayed in ACCs [25, 26]. Washout can be expressed as absolute or relative, of which the absolute washout allows for better discrimination [27]. Adenomas are typically associated with an absolute washout of $> 60\%$ (sensitivity 86–100%, specificity 83–92%) at 15 min after contrast administration [27]. An important consideration is that all mentioned features are suggestive for malignancy, but definitive clinical diagnosis of an ACC can only be made in the presence of metastatic disease. In clinical practice, it is recommended to perform both unenhanced and contrast-enhanced CT for initial assessment and follow-up. The general consensus is that surgery is recommended in tumors larger than 6 cm [28], although exceptions exist if all other characteristics point toward a benign lesion. In tumors with several suspicious imaging features, further evaluation is warranted. For lesions smaller than 4–6 cm, surveillance with repeated imaging is important although there is no consensus yet on the duration of follow-up. It is generally not recommended to use fine needle aspiration biopsy (FNAB) in ACC, because of the risk on hemorrhage, tumor rupture, spill and the limited diagnostic value.

Recent advances regarding imaging of adrenal lesions have focused on ¹⁸F-fluorodeoxyglucose positron emission tomography (¹⁸F-FDG PET) to differentiate ACCs from ACAs [29]. It is based on the fact that malignant adrenal tumors show a higher uptake of radiolabeled glucose compared to normal and adenomatous adrenal tissue. It remains to be elucidated whether quantitative assessment, using standardized uptake values (SUV), or qualitative assessment by visual inspection, has greater diagnostic accuracy. In a meta-analysis including 1217 patients, a mean sensitivity of 97% and specificity of 91% was found for diagnosing adrenal malignancy with ¹⁸F-FDG PET [29]. No difference was found between ¹⁸F-FDG PET and ¹⁸F-FDG PET/CT, although it is hypothesized that the use of PET/CT performs better compared to PET, as functional information and anatomical details can be combined. It should be noted that false-negative and false-positive findings have been described, due to small size of the lesion (<1 cm), or increased uptake by several benign conditions, like lipid-poor adenomas, respectively [29]. Leboulleux et al. showed that PET/CT was complementary to total-body CT in detection of metastasis [30]. A recent study proposed the postoperative use of ¹⁸F-FDG PET as second-line imaging modality following a CT with suspicion of recurrence [31].

Another PET tracer is ¹¹C-labeled metomidate (MTO), which binds with high affinity to CYP11B enzymes. It specifically identifies the adrenocortical origin of an adrenal lesion [32]. A study combining MTO-PET scan results, histopathology, and hormonal secretion showed that MTO-PET could differentiate adrenocortical lesions from pheochromocytomas and metastasis with a sensitivity of 89% and a specificity of 96% [33].

As an alternative, [¹²³I]IMTO for single photon emission computed tomography (SPECT) also shows high and specific uptake in adrenocortical tissue [32, 34]. Patients with high uptake of this tracer are also potential candidates for treatment with radionuclide therapy with [¹³¹I]IMTO, since this is specifically taken up by the tumor [35].

Proton MR spectroscopy, using the choline-creatinine ratio, has shown to discriminate pheochromocytomas and adrenal adenomas from adrenal metastasis and ACC with a sensitivity of 92% and a specificity of 96% [36]. Although all new imaging techniques seem promising, external validation needs to be performed to determine their clinical utility.

Staging

The staging system proposed by ENSAT is currently used to stage ACC and is suggested to accurately correlate with patient outcome (Table 12.2) [37]. This was confirmed in the independent SEER cohort [38]. Stages I and II are characterized by localized ACCs with a size of ≤5 cm, or >5 cm, respectively. Stage III includes tumors with infiltration in regional lymph nodes or surrounding tissue (e.g., para-adrenal adipose tissue or adjacent organs) or a tumor thrombus in the vena cava and/or renal vein. ACCs with distant metastasis are categorized as stage IV. Of all stages, most patients present with stage IV disease [38].

Pathology

The Weiss score (WS) consists of nine histopathological parameters of proliferation, nuclear abnormality, and tumor extension [39]. An adre-

Table 12.2 European Network for the Study of Adrenal Tumors staging system

ENSAT stage	
I	T1, N0, M0
II	T2, N0, M0
III	T1-2, N1, M0 T3-4, N0-1, M0
IV	Any M1

Tumors are classified as follows: *T1* tumor ≤5 cm, *T2* tumor >5 cm, *T3* histologically proven tumor infiltration into surrounding (fat) tissue, *T4* tumor invasion of adjacent organs or venous tumor thrombus in vena cava or renal vein, *N0* absence of positive lymph nodes, *N1* positive lymph node(s), *M0* no distant metastasis, *M1* presence of distant metastasis (From Fassnacht et al. [37], with permission)

nocortical tumor with a WS of ≥ 3 criteria present in the tumor is considered as malignant. However, the accuracy of the WS is less clear in tumors with a WS of 2 or 3, because they may have variable malignant behavior and are often referred to as tumors with undetermined behavior. The WS is thereby difficult to apply in adrenocortical tumor variants like myxoid-, sarcomatoid-, or mixed variants. Duregon et al. recently reported the most important challenges regarding diagnostic pathology: adrenocortical tumors versus nonendocrine tumors, cortical versus medullary tumors, and ACCs versus ACAs [40]. Interobserver variability, the lack of reproducibility, and high intratumor heterogeneity make the reappraisal by a second independent expert pathologist mandatory [40]. Several other scorings systems have been proposed since the introduction of the WS [41, 42], but to date the WS is the cornerstone of pathological diagnosis of ACC.

Other molecular markers (e.g., IGF2 staining, Ki67, reticulin staining) have also been evaluated for distinguishing ACCs from ACAs [43]. A reticulin algorithm, containing the disruption of reticular networks with at least one of the following three parameters, necrosis, high mitotic rate, or vascular invasion, may specifically be applicable for cortical tumor variants like oncocytic and myxoid subtypes [44–46]. Further details about adrenal pathology are described in Chap. 2.

Urinary Metabolomics

Urinary steroid profiling, based on excessive amounts of adrenal steroids produced by the tumor, has been introduced as a sensitive method to diagnose ACC and to detect recurrence, progression, and treatment response [47]. In a study including 102 ACAs and 45 ACCs, urinary steroid profiling differentiated ACCs from benign tumors with a sensitivity and specificity of 90% [48]. The 11-deoxycortisol metabolite tetrahydro-11-deoxycortisol was the most accurate marker. Kerkhofs et al. found a sensitivity of 100% and specificity of 99% for discriminating ACCs from other adrenal disorders using this method [49].

Treatment

For localized ACC, successful tumor-directed surgery (R0 resection) is the only potentially curative treatment for ACC (see Chap. 13). However, even after complete resection, recurrence rates are high (30–50%) and are even higher in patients with incomplete resection [9, 37, 50–53]. Adjuvant treatment aims to decrease these high recurrence rates. For patients with severe hormone excess who cannot be controlled otherwise or in patients with a limited number of organs with tumor metastases (≤ 2) involved and a resectable tumor mass, debulking surgery can also be beneficial [54]. When this is not the case, medical therapy should be started as soon as possible. Figure 12.1 shows the current therapeutic management approach in patients with either localized or metastatic ACC [55–57].

Surgery

To assure an optimal outcome and to prevent tumor spillage and incomplete resection, surgery should only be performed by an experienced and high volume surgeon (>15 adrenalectomies per year). Postoperative infectious complications appear to be an independent predictor of decreased overall survival (OS) and should be prevented [58]. In patients with CS, postoperative glucocorticoid replacement therapy is necessary. A thorough endocrine and imaging workup is recommended to guide the surgical approach, since the choice of the best surgical approach is still controversial. A recent systematic review concluded that open adrenalectomy with lymph node dissection should be regarded as standard treatment for ACC [59]. In patients with stage I-II disease and a tumor diameter $< 8–10$ cm, laparoscopic resection may be performed. Another key question is the clinical utility of lymph node dissection. A detailed description about surgery for patients with adrenocortical tumors can be found in Chap. 13.

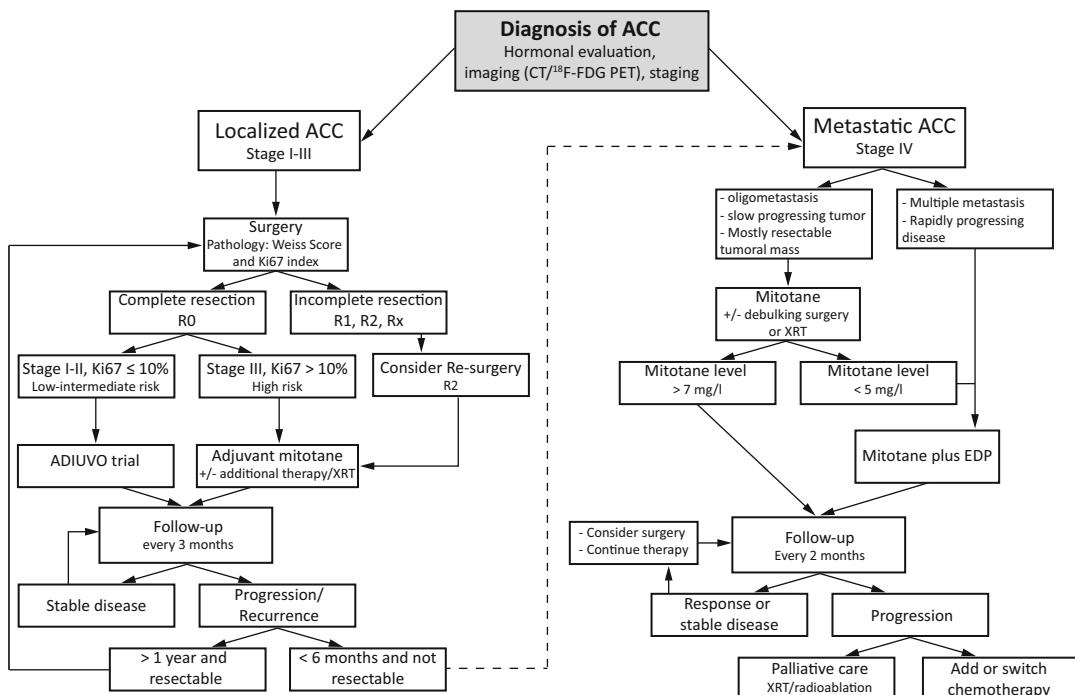


Fig. 12.1 Flow chart of management for patients with ACC. Debulking surgery should only be performed in patients with complaints due to severe hormone excess, in patients with ≤ 2 involved organs, and a resectable tumoral mass. ACC adrenocortical carcinoma, CT computed tomography; ¹⁸F-FDG PET ¹⁸F-fluorodeoxyglucose

positron emission tomography, *Ki67* proliferative index, *R1* incomplete resection with positive microscopic margins, *R2* incomplete resection with positive gross margins; *Rx* unknown margin status, *XRT* radiotherapy, *EDP* etoposide doxorubicine and cisplatin [55–57]

Mitotane Treatment

Mitotane [1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane (*o,p'*-DDD)] was first described to have therapeutic effects on the adrenal cortex in 1949 [60]. To date, it is the only approved drug for treatment of ACC [61] and is thought to primarily act by disruption of mitochondria subsequently activating an apoptotic process [62]. Endoplasmatic reticulum stress activation was also identified as a key mechanism of action by mitotane [63]. It also has inhibitory effects on steroidogenesis enzymes, as described as follows.

Management of Mitotane Treatment

There are several challenges regarding management of treatment with mitotane. Side effects in different organ systems can be severe, limiting long-term clinical tolerance. Treatment should

thereby be guided by an experienced physician. Mitotane is often accompanied by gastrointestinal side effects, like nausea, vomiting, anorexia, and diarrhea [64, 65]. These symptoms can be reduced by taking mitotane with food, treatment with anti-emetic and antidiarrheal medications, or distributing the mitotane amount in smaller dosages during the day. Other common side effects are neurologic and include fatigue, dizziness, ataxia, decreased memory, or depression [64, 65]. This group of symptoms most frequently causes withdrawal of the drug. Hypercholesterolemia, hypouricemia, and hepatotoxicity can also occur. Mitotane may also inhibit thyroid function (TSH and free T4), mimicking central hypothyroidism [8, 64, 66]. Thyroid function tests should be monitored regularly and particularly when patients present with fatigue, thyroxine replacement therapy should be considered when free T4 levels drop below normal.

The target plasma concentration of mitotane is 14–20 mg/L and monitoring is of great importance. In several studies, it has been shown that patients who reached this target concentration present with less recurrences and show a prolonged recurrence-free survival [67–70]. Different dosing regimens are proposed, aiming to decrease long-term toxicity, and increase efficacy and patient compliance. For example, it has been shown that a high-dose starting regimen does not lead to different mitotane levels nor a different rate of adverse events, compared to a low-dose starting regimen [71]. Independent of the dosing strategy, it is recommended to measure mitotane blood levels every 2–3 weeks in the first 3 months, and after this period every 4–6 weeks [55].

In addition to the adrenolytic effect of mitotane, mitotane inhibits several steroidogenic enzymes, like cholesterol side chain cleavage enzymes, CYP11A1, and CYP11B1, leading to hypocortisolism [72, 73]. Hydrocortisone replacement is therefore mandatory during mitotane treatment. Another important consideration is mitotane-induced CYP3A4 activity, which can lead to important drug interactions [74]. This CYP3A4 induction results in an enhanced metabolism of cortisol and plays, together with induction of cortisol binding globulin (CBG) and the adrenolytic effect of mitotane on the contralateral adrenal, a role in the occurrence of hypocortisolism [75–77]. Hydrocortisone substitution during mitotane treatment should therefore be given in higher dosages. Mitotane can also increase SHBG concentrations and thereby its binding capacity to 17 beta-hydroxysteroid hormones (e.g., testosterone). Along with inhibition of 5 α -reductase, this may lead to clinical signs of hypogonadism in male patients [77–79]. In this case, testosterone replacement therapy is recommended, although this is relatively ineffective due to the increase in SHBG levels.

Adjuvant Mitotane Treatment

The high recurrence rates after surgery provide the rationale for adjuvant treatment of ACC. Mitotane is currently used as a first-line treatment for patients with metastatic ACC and is

also frequently used in the adjuvant setting. Generally, mitotane is started within 3 months after surgery with a dose of 1.5 g/day. The dose should be increased every 4–6 days by 0.5–1 g/day until a daily dose of 6 g is reached [55]. After 3 weeks, dosage should be adjusted according to tolerability and blood levels of mitotane. Maximum dose is 12 g/day, but most patients do not tolerate >8 g/day. In higher risk patients, mitotane is increased more rapidly. The duration of treatment varies from 2 to 5 years.

The efficacy of mitotane as adjuvant treatment modality for patients with ACC has been only investigated retrospectively and studies report discordant results [43]. Three studies (total $n=919$) have shown prolonged disease-free survival for mitotane-treated patients [10, 52, 80]. The best evidence was provided by a study performed by Terzolo et al. In this combined Italian and German study, the median recurrence-free survival was 42 months for the mitotane-treated patients versus 10 months in the Italian control group and 25 months in the German control group [80]. There was no effect on overall survival. In contrast to these findings, several other studies, including a recent one by Beuschlein and colleagues, reported no effect on disease-free survival [81]. Currently, adjuvant mitotane treatment is recommended especially in patients with high recurrence risk postsurgery (i.e., stage III, Ki67>10%, R1 or Rx resection) [55]. Several centers recommend mitotane treatment in all patients with ACC after surgery. The ADIUVO study, a phase 3 trial, is now being conducted to address the need for adjuvant mitotane treatment in patients who undergo R0 resection and have low-to-intermediate recurrence risk (stage I-III, Ki67≤10%).

Mitotane for Advanced Disease

For patients with unresectable or metastatic ACC, all therapies should be considered palliative. The first-line treatment option is mitotane. Thirty-three percent of the patients treated with mitotane either obtain complete response, partial response, or stable disease [43]. A subgroup of patients also has very slow disease progression while on mitotane therapy. There is unfortunately

still limited knowledge about the patient or tumor-related factors that predict response to mitotane. It is important to rapidly titrate the mitotane dose, if tolerated, to target serum levels between 14 and 20 mg/L in patients with advanced disease. In case the mitotane concentration in the blood is still below 7 mg/L after 3 weeks of treatment, or in case of progression of advanced disease, a combination of mitotane with chemotherapy can be considered (see Section “Chemotherapy”).

Predictive Markers for Mitotane

Mitotane treatment is only effective in a subset of patient but is associated with severe toxicity. Several factors have been identified that are associated with mitotane sensitivity, which may be helpful in the prediction of treatment response. High protein expression of CYP2W1, independent of ENSAT stage, has been associated with a longer overall survival and time to progression in patients treated with mitotane. This difference in survival was not significant in patients who only underwent follow-up [82]. Low expression of the ribonucleotide reductase large subunit 1 (*RRM1*) gene, and high sterol-O-acyl-transferase 1 (SOAT1) expression correlated with a better response to mitotane treatment, as measured by prolonged time to progression for SOAT1 and a prolonged disease-free survival and overall survival for *RRM1* [63, 83]. As a possible mechanism of the involvement of the *RRM1* gene in mitotane sensitivity, Germano et al. showed that silencing of the *RRM1* gene by small interfering RNA increased the intracellular concentration of mitotane [84].

Chemotherapy

Although several cytotoxic drugs have been studied in advanced ACC, only a few large trials have been performed. Mitotane is thought to enhance sensitivity of ACC cells to cytotoxic drugs by inhibiting the *MDR1* gene, which is a gene associated with the chemoresistant character of ACCs [85–87]. However, it is likely that other factors determine sensitivity to chemotherapeutics as

well, since combination therapy with chemotherapy and mitotane is still not very effective. An increase in mitotane dose is frequently needed in patients concomitantly receiving chemotherapy, probably due to malabsorption.

The first randomized trial showed that for patients with advanced ACC, a combination of mitotane with etoposide, doxorubicine, and cisplatin (EDP-M) had a longer median progression-free survival as compared to patients receiving streptozotocin and mitotane (5.0 vs. 2.1 months) [88]. No effect on overall survival was observed. Although the median overall survival is only 14.8 months, this regimen (EDP-M) is the preferred choice in case of progression of advanced disease after mitotane monotherapy. Other possible chemotherapeutic options if patients fail EDP-M are gemcitabine and capecitabine, thalidomide, or metronomic cytotoxic regimens [89–92]. Since cytotoxic therapies are not effective in all patients, research is focusing on factors associated with sensitivity and identifying patients who are more likely to respond. High protein expression of the excision repair cross complementing group 1 (ERCC1) is thought to be a predictor of response, since it was associated with a worse overall survival in ACC patients treated with platinum-based chemotherapy [93].

Radiotherapy

Radiotherapy can also be used as adjuvant treatment for ACC. However for decades, ACC was considered a radiotherapy-resistant cancer and studies reported poor and contradictory results of postoperative radiotherapy [56]. More recently, several studies have shown a role of radiotherapy in improved control of local disease postsurgery (56–100% of patients had local control), although no effects on disease-free and overall survival were found [94–98]. Due to the retrospective design in these studies, it is difficult to determine the reasons for the discordant results. Therefore, prospective studies are required to establish the value of adjuvant radiotherapy for local disease control. Another indication for radiotherapy may be for palliation in patients with symptoms.

Several studies, although all had very small cohorts, have suggested a response as defined as pain relief after radiation in patients with ACC [94, 98].

Treatment of Hormone Excess

Control of excess hormone levels is important to control symptoms and to improve quality of life, and in some cases it can even improve survival rates. Mitotane is effective for lowering serum cortisol levels by the mechanisms mentioned earlier. Ketoconazole and metyrapone, alone or in combination, are also commonly used to control glucocorticoid excess [99]. Ketoconazole is usually administered with a starting dose of 200 mg twice daily, which can be increased to 1200 mg/day. Metyrapone has a starting dose of 250 mg twice daily and can be increased to 2–3 g/day administered in intervals of 250 mg. Liver enzymes should be carefully monitored during ketoconazole treatment and levels of adrenal androgens and mineralocorticoid precursors may increase under metyrapone treatment [100, 101]. Mifepristone is a direct receptor antagonist used in case of glucocorticoid excess, with hypokalemia and hypertension as the most prevalent side effects of mifepristone [100–102]. Mifepristone treatment can be initiated with 300 mg daily and titrated up to 1200 mg daily. Spironolactone (doses up to 200–400 mg/day) can also be used to control androgen-related effects in women with androgen-secreting ACCs and mineralocorticoid effects in patients with mineralocorticoid-secreting tumors [103].

Targeted Therapies

New insights in molecular and genetic alterations underlying ACC pathogenesis have led to the identification of several potential therapeutic targets. Insulin-like growth factor-2 (IGF2) binding to the IGF1R activates the PI3K/Akt/mTOR pathway. Since IGF2 overexpression is the most important molecular alteration in ACCs [104–107], the IGF-mTOR pathway has become one of

the main focuses for targeted therapy development. However, the various clinical studies with IGF1R monoclonal antibodies and IGF1R inhibitors have only shown positive results in a small subset of patients [43, 108–110]. The first phase III trial with Linsitinib (OSI-906) showed partial response or stable disease in only 9/90 patients over a period of 24 weeks [111]. IGFs are also known to activate escape mechanisms from mTOR inhibitors. To circumvent this, a phase II study combined the IGF1R monoclonal antibody cixutumumab, with the mTOR inhibitor temsirolimus, which resulted in prolonged (6–21 months) stable disease in 11 of 26 patients [110].

Several other growth factors have been implicated in ACC, such as transforming growth factor- α (TGF- α) [112], and vascular endothelial growth factor (VEGF). VEGF, together with its receptor VEGFR, are overexpressed in ACC [113–115]. In a phase II trial investigating the efficacy of axitinib (VEGFR tyrosine kinase inhibitor) in patients with advanced ACC, 8/13 patients had stable disease for 3 months [116]. Many receptors of the pathways involved in ACC belong to the tyrosine kinase family, possibly making multityrosine kinase inhibitors (TKI) more efficient. Sunitinib, a multi-TKI, resulted in stable disease in 5/35 patients [117]. Discouraging results were obtained from clinical trials with several other multi-TKI [118–121]. The WNT signaling pathway also plays an important role in sporadic adrenocortical tumorigenesis [122]. Targeting this pathway was only effective in vitro [123]. Clinical trials have not yet been performed.

In conclusion, only a subset of patients appear to benefit from targeted therapies in ACC. We do have to acknowledge that these trials include pre- and co-treated patients, which could lead to important drug interaction with, for example, mitotane. In addition, there is evidence that monotherapy with TKIs causes compensatory activation of other signaling pathways [124]. Because of the lack of efficacy in clinical trials investigating targeted monotherapies, the general view is that combination therapy is potentially more effective to have meaningful tumor response in patients with ACC. Thus, an important

challenge is identifying the subpopulations of patients who do benefit from specific therapies.

Follow-Up

Patients with ACC should generally be followed every 3 months after diagnosis of localized disease, and every 2–4 months for advanced or metastatic disease. After a recurrence-free time of 2 years, surveillance intervals can be gradually increased [55]. However, follow-up is recommended, even in patients without evidence of disease, over a minimum period of 10 years. Patients should undergo a complete physical examination, biochemical evaluation, and imaging workup (abdominal CT or MRI and thoracic CT) [55].

Prognostic Factors

Although the general prognosis of patients with ACC is poor, the disease course can be very heterogeneous. Accurate prognostication is critical for patients and treating physicians in order to guide decisions regarding treatment, and intensity and duration of follow-up. The following prognostic factors have been proposed.

Pathology

Among the pathological parameters, the ENSAT stage at initial diagnosis and complete resection (R0) are the most important and validated prognostic factors for patients with ACC [37, 38, 55]. Five year survival rates range from 70 to 85 % for stage I, 60–65 % for stage II, 30–55 % for stage III, and 10–15 % for stage IV [2, 37, 38]. Since advanced and metastatic disease largely determines the prognosis, all important and generally available prognostic factors for ACC stage I/II are summarized in Table 12.3. In the largest study to date, assessing several clinical and histopathological criteria after complete resection (R0), Ki67 index was identified as the single most important factor predicting recurrence [81]. This is not the first study to report

the prognostic value of Ki67 in patients with ACC [125–127]. Ki67 is a routine assay at most medical centers for diagnosis and prognostication, and also for guiding treatment decisions [1, 57]. A recent study investigated the reproducibility of Ki67 staining [128]. The authors showed that visual estimation of Ki67 labeling results in high interobserver variability and computerized digital image analysis seems to be a reliable alternative [128]. In a recent large ENSAT study, including 444 patients with stage III or IV ACC, several factors appeared to be important for prognostication, namely, a modified ENSAT classification (III, IVa, IVb, IVc), tumor grade, resection status, age, and tumor- or hormone-related symptoms [50]. Tumor grade included the Weiss score and Ki67 index. The association between complete resection and a better prognosis has also been reported by Bilimoria et al., who showed that the median survival decreased from 51.2 months after complete resection (R0) to 12.6 months following incomplete resection with microscopic positive margins (R1) and 7.0 months after incomplete resection with gross positive margins (R2) [129]. The Weiss score has also been associated with prognosis; however, findings are inconsistent between different studies. Mitotic activity has been reported as the most significant determinant of survival [127, 130].

Table 12.3 Widely available prognostic factors for patients with ENSAT stage I/II adrenocortical carcinoma

Factor	Poor prognosis
ENSAT stage	II
Ki67	>10 %
Weiss score	>6
Tumor size	≥12 cm
Resection status	R1, R2, Rx
Mitotic rate	>5 per 50 high-power fields (HPF)
Hormone secretion	Cortisol secretion

ENSAT European Network for the Study of Adrenal Tumors, *Ki67* proliferative index, *R1* incomplete resection with positive microscopic margins, *R2* incomplete resection with positive gross margins, *Rx* unknown margin status

Clinical Features

The impact of hormone secretion by ACCs on outcome is not entirely clear. The exact mechanism also remains to be elucidated, although it is known that overt CS represents an important cause of postsurgical and postchemotherapy morbidity, due to an increased risk of infections, and metabolic and vascular complications. Several studies consistently showed a decreased overall survival in patients with cortisol secreting ACCs [17, 131, 132], and one study also reported an increase in recurrences [132]. However, not all studies report evidence that the functional status of the tumor influences outcome [7, 15]. Interestingly, in a study by Abiven et al., mitotane treatment only prolonged survival in patients with cortisol-secreting tumors, whereas this was not the case for the whole study population [17]. The same tendency was reported 1 year later, suggesting that patients with cortisol-producing tumors obtain more benefit from mitotane treatment [133]. Berruti et al. also found a decreased overall survival and recurrence-free survival for patients with cortisol-secreting tumors, but did not find an interaction between cortisol secretion and mitotane treatment [10]. Recently, a study including 234 patients with ACC did show that patients with cortisol-secreting tumors had a higher risk of perioperative complications and a 67% higher risk of recurrence on long-term follow-up [53]. An older age has also been associated with an adverse prognosis [7, 17], but data are inconsistent [80, 134].

Since these studies are population based, it is difficult to expand these findings to the individual patient. Therefore, Kim et al. studied 148 patients with curative resection of ACC and proposed normograms to stratify patients into distinct prognostic groups [135]. Normograms for recurrence-free survival consisted of tumor size ($</\geq 12$ cm), nodal status, T stage, cortisol secretion, and capsular invasion, whereas factors included for overall survival were tumor size ($</\geq 12$ cm), nodal status, and resection margin [135]. Further studies are needed to validate these findings.

These data together highlight the importance of a tailored surveillance of patients postoperatively, based on patient and tumor characteristics, such as the cortisol secretion status of the tumor.

Molecular Characteristics

Several molecular markers have been proposed for prognostic classification of ACC patients. It is suggested that differences in survival between patients with ACC can be predicted based on analyzing gene expression profiles (transcriptome). Using this technique, two subgroups of ACCs have been identified: cluster C1A and cluster C1B, with a remarkably worse outcome in cluster C1A [104, 136–138]. Cluster C1A could be further divided into three subgroups, with inactivating *TP53* mutations (C1A-p53), activated β -catenin (C1A- β -catenin), and one group with a still unidentified molecular alteration (C1A-x) [139]. Epigenetic modifications (e.g., DNA methylation and microRNAs) also appear to be different in the C1A and C1B groups and may also be informative for prognostication in ACC patients [140]. Barreau et al. made the first correlation of DNA methylation with survival outcome in patients with ACC [141]. A CpG island methylation phenotype (CIMP) was defined as having a higher methylation compared to ACAs. The CIMP group could further be divided into CIMP-high and CIMP-low, of which the high group was associated with a poor prognosis [141]. Remarkably, the C1A-p53 and C1A-x subgroups with poor prognosis showed a CIMP profile, whereas the C1A- β -catenin and the good-prognosis (C1B) group showed a non-CIMP profile.

More specifically, expression levels of several genes have been correlated with clinical outcome in ACC, like the steroidogenic factor 1 (SF1), matrix metalloproteinase type 2, glucose transporter GLUT1, pituitary tumor transforming gene 1 (PTTG), and the transforming growth factor β signaling mediator SMAD and the transcription factor GATA-6 [142–146].

Pediatric Adrenocortical Carcinoma

Adrenocortical carcinomas in the pediatric population are histologically and clinically different from those in the adult population, even though there is a certain overlap. The Surveillance Epidemiology End Results data indicate an incidence of 0.21 per million population per year below the age of 20 years, of which over 50 % occurs under the age of 5 [147–149]. When hormone replacement therapy became available, outcome of children with ACC dramatically improved due to prevention of postoperative acute adrenocortical insufficiency [150]. Pediatric ACC frequently occurs sporadically, but in 50 % of cases predisposing constitutional genetic factors have been found, like the Beckwith–Wiedemann and Li–Fraumeni syndrome [5, 151]. Consequently, it is important to investigate possible syndrome associated germline TP53 mutations in children presenting with ACC [152]. Children more frequently present with signs of androgen excess compared to adult patients (84 % of the cases), while CS is infrequent (6 %) [7, 153, 154]. Functional tumors may be indicative for a poor prognosis in adults, but this has not been found in children [148]. The behavior of pediatric ACC is more difficult to predict compared to adult ACCs, and prognosis is thought to be quite good when patients have survived the first few years after diagnosis [155, 156]. Staging is performed in 3 distinct groups: completely resected small tumors with excellent prognosis, completely resected large tumors, and residual or distant metastatic disease [154]. The most important prognostic factors are largely similar to adult patients with ACC: tumor size, resection status, several variables included in the Weiss score (capsular invasion, vascular invasion, necrosis, and increased mitotic activity), and stage [148, 149]. As with adult ACCs, the Weiss score can be troublesome in pediatric tumors. In 1994, Bugs and colleagues proposed a modified Weiss system, identifying adenomas, low-grade carcinomas, and high-grade carcinomas, since in general two distinct prognostic groups can be recognized in pediatric adrenocortical tumors [157]. High-grade carcinomas and tumor weight were the most reliable prognostic

factors. It is known that younger patients have a better outcome compared to older children with ACC [147, 154, 158]. In pediatric ACCs, surgery is also the single most important procedure in successful curative treatment. Efficacy of mitotane, chemotherapy, and radiation is still controversial and further studies are needed.

Conclusions and Future Perspectives

Significant efforts that have been made for expanding our knowledge regarding the pathogenesis and treatment of ACC have resulted in some progress in the management of ACC. Due to its rarity, several collaborative efforts, like the European Network of the Study of Adrenal Tumors (ENSAT), have emerged and have made important contributions. Continued collaborations and large consortia will be necessary to improve the diagnosis and therapeutic strategy for patients with ACCs. Studies focused not only on new compounds and targets for therapy, but also on improving and studying the indications for already existing therapeutic options, like mitotane. Although mitotane remains the only approved drug, molecular and genetic studies have paved the way to trials with several targeted drugs. To date however, these drugs have only shown efficacy in a subset of patients. This emphasizes the importance of identifying predictive markers in order to prevent overtreatment and unnecessary side effects. Future studies should also try to find markers to more accurately determine the risk of recurrence and better classification of patient prognosis. Currently, it is important to approach every patient based on their clinical and tumor characteristics in order to be able to choose the optimal diagnostic and therapeutic strategy, and postoperative surveillance.

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Dhaval Patel

Indications

The evaluation of an adrenal mass requires a stepwise approach to determine whether any type of surgical or medical intervention is necessary. The initial questions in the evaluation of an adrenal mass are to determine whether it is functional or nonfunctional and whether it is benign or malignant. If the adrenal mass appears to be malignant, then the clinician must determine whether it is a primary adrenocortical tumor or a metastatic tumor from another site such as lung cancer, renal cell carcinoma, and breast cancer. The indications for adrenalectomy are listed in Table 13.1. The workup for each of these conditions is explored in detail in other chapters of this book with respect to functional tumor and/or malignancy. Herein the operative approach, technique, extent of resection, and outcome of surgical intervention will be discussed.

Approaches and Techniques

The surgical approach to the resection of adrenal neoplasms includes open, laparoscopic, or robotic approaches. The surgeon can perform these operations either through a transabdominal or retroperitoneal approach. Minimally invasive surgery has been shown to be associated with significant advantages compared to open resection for patients with benign adrenal disease including shorter hospital length of stay, decreased morbidity including pulmonary, renal and cardiac complications, and decreased pain [1]. When patients are suspected of having adrenocortical carcinoma either preoperatively or intraoperatively, the most prudent course of action is to perform an open *en bloc* resection of the adrenal gland and any involved organs. Mir and colleagues compared outcomes of patients with adrenocortical carcinoma at a single high volume center with either a laparoscopic or open approach. Patients who underwent a laparoscopic adrenalectomy had a nonstatistical trend for increased recurrence and death [2]. Donatini and colleagues, however, advocate that laparoscopic adrenalectomy in patients with adrenocortical carcinoma can have similar oncologic outcomes to open adrenalectomy with proper patient selection. Patients with tumors less than 10 cm, and no evidence of extra-adrenal extension were ideal candidates for laparoscopic adrenalectomy.

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Table 13.1 Indications for surgery

Functional tumors	Nonfunctional tumors
Pheochromocytoma	Incidental adrenal mass greater than 4 cm or increasing in size
Conn's syndrome with lateralization by adrenal venous sampling (aldosteronoma or hyperplasia)	Isolated metastasis from another primary site
Cushing's syndrome	Symptomatic cyst or angiomyolipoma
Cortisol secreting adenoma	Adrenocortical carcinoma
Bilateral adrenal hyperplasia refractory to medical therapy	
Ectopic adrenocorticotrophic hormone (ACTH) syndrome	
Cushing's disease with failed transsphenoidal surgery	
Virilizing/feminizing tumors	
Adrenocortical carcinoma	

Disease-specific survival and disease-free survival did not differ between the two groups [3]. Although these reported findings may indicate the value of a minimally invasive approach, the current standard of care is open adrenalectomy for patients with adrenocortical carcinoma, since the risks of tumor spillage greatly outweigh the benefits of a laparoscopic approach.

Anatomic and Technical Considerations

The adrenal glands are located within the retroperitoneum and are superior to the kidneys. The paired glands lie within the retroperitoneal fat, which can be quite extensive in patients with Cushing's syndrome. The surgeon should be familiar with the venous drainage of the adrenal glands, which differs between the left and the right adrenal gland. The right adrenal vein, typically, drains directly into the posterior lateral aspect of the inferior vena cava. The left adrenal gland vein joins the inferior phrenic vein and then drains into the left renal vein. Pheochromocytomas and adrenal malignancies, however, may have large accessory veins requiring ligation. It has

been reported that 13 % of patients may have variant venous anatomy. Scholten and colleagues retrospectively analyzed a large cohort of patients and found that 70 out of 546 adrenalectomies had variant venous anatomy. The incidence of variant venous anatomy was higher on the right side compared to the left side (17 % vs. 9 %). In addition, patients with pheochromocytomas (35 % vs. 21 %) and patients with larger tumors (mean 5.1 vs. 3.3 cm) were more likely to have variant venous anatomy defined as no main adrenal vein identifiable, 1 main adrenal vein with additional small veins, 2 adrenal veins, and more than 2 adrenal veins, and variants of the adrenal vein drainage to the inferior vena cava and hepatic vein or the inferior phrenic vein [4]. Comparatively, the arterial blood supply is similar for both adrenal glands and consists of superior, middle, and inferior adrenal arteries.

Resection of the adrenal gland is done under general anesthesia, and those patients who are preoperatively planned for an open resection may benefit from an epidural catheter placement for postoperative pain control. All patients should receive deep venous thrombosis (DVT) prophylaxis. Prophylaxis is especially important in patients with Cushing's syndrome, since the risk of venous thromboembolism has been documented and reported to be more than tenfold higher compared to the general population [5]. Patients with biochemical evidence of either Cushing's syndrome or subclinical Cushing's syndrome should receive perioperative stress dose steroid including 100 mg of hydrocortisone preoperatively followed by a quick 3-day taper [6]. A foley catheter should be placed. Patients with pheochromocytomas may require further monitoring due to hemodynamic changes despite adequate alpha and/or beta blockade. Invasive monitoring should include an arterial line and a central venous catheter. Vasoactive medications should be readily available in case of hypo- or hypertension. In the case of patients undergoing surgery for malignancy requiring large *en bloc* resections, invasive monitoring may be required. Patients with adrenocortical carcinoma, especially right-sided tumors, may require vascular grafts and instruments available for reconstruction.

Preoperative, surgical evaluation of inferior vena cava thrombus extension is of the utmost importance for surgeons operating on patients with adrenocortical carcinoma. Preoperative preparation for common adrenal tumors is presented in Table 13.2.

Transperitoneal Laparoscopic

Transabdominal laparoscopic adrenalectomy is currently the most common laparoscopic approach used by most surgeons. The patient is placed in a lateral decubitus position with the anterior superior iliac spine aligned with the point of flexion of the operating room table. The patient is secured to the operating room table with the table flexed. The midclavicular line is identified and an incision is placed one finger breadth below the costal margin. A zero degree videoscope is used to enter the peritoneal cavity under direct visualization. The zero degree videoscope is exchanged for a 30° scope and additional trocars are placed under direct visualization. Trocar sites are shown for a laparoscopic left adrenalectomy in Fig. 13.1.

For a right adrenalectomy, four trocars are placed under direct vision. The trocars are placed from the midclavicular line to the mid-axillary line with two fingerbreadths between the trocars.

The first step in dissection begins with the division of the triangular ligament of the liver to mobilize the right lobe of the liver medially and expose the right adrenal gland. Upon completion of this step, a snake, fan, or paddle retractor is placed through the medial most port on the posterior aspect of the right lobe of the liver. Retraction of the right lobe of the liver creates a working space. The dissection from the triangular ligament is identified and brought down inferiorly by dividing the peritoneum and Gerota's fascia along the liver, lateral to the inferior vena cava. During this step, manipulation of the tumor should be kept to a minimum. Grasping of the adrenal gland and/or tumor should be avoided to reduce the possibility of tumor capsule violation. The dissection is continued in the space between the inferior vena cava and the right adrenal gland. The right adrenal vein is commonly encountered during this top-down dissection.

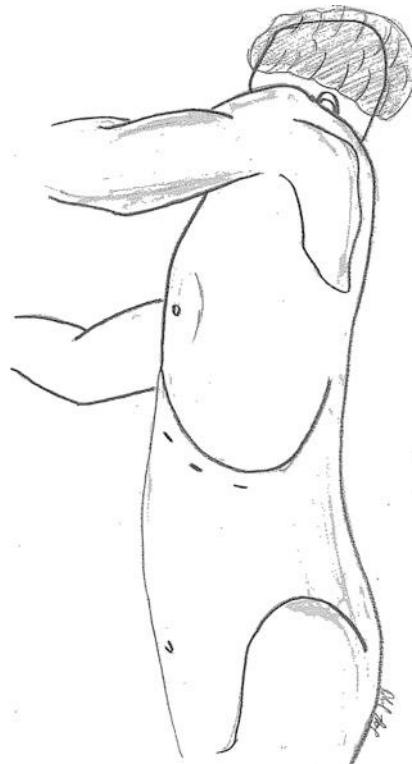


Table 13.2 Preoperative preparation

Tumor type	Preoperative preparation
Pheochromocytoma	Alpha receptor blockade, hydration Add beta blocker if tachycardia
Aldosteronoma	Replete hypokalemia, blood pressure control
Cushing syndrome	Perioperative stress steroids
Adrenocortical carcinoma	Perioperative stress steroids if cortisol secreting Replete hypokalemia, blood pressure control if aldosterone secreting
Incidentaloma (nonfunctional)	None

Fig. 13.1 Patient positioned in a lateral decubitus position for a planned left adrenalectomy. The three trocar sites are marked on the patient

Once the vein is identified, it is circumferentially dissected, clipped, and divided. There may be accessory veins and/or arteries, which should be clipped and divided. The inferior pole of the gland is dissected from the renal hilum, avoiding injury to the renal artery and vein. The attachments of the gland to the superior pole of the kidney and posterior musculature are divided. The dissection is extended up to the diaphragm with removal of the periadrenal fat. Once the final attachments to the diaphragm are transected, the adrenal bed is examined for bleeding. The specimen is removed through the lateral most port in a bag. The steps of a right adrenalectomy are depicted in Fig. 13.2.

For a left adrenalectomy, three trocars are placed under direct vision. The trocars are placed from the midclavicular line to the midaxillary line approximately two fingerbreadths apart. The lateral attachments of the spleen are transected approximately 1 cm from the lateral edge of the spleen. This dissection is completed from the splenic flexure of the colon to the superior pole of the spleen with visualization of the gastric fundus. Upon completion of this step, the spleen and the pancreas will be retracted medially by gravity. Similar to the right adrenal, dissection is performed top-down and carried down posteriorly to the psoas muscle. Often, the inferior phrenic vein can be identified during the medial dissection and traced down to the left adrenal vein. Once the left adrenal vein is identified, it is circumferentially dissected, clipped, and divided above the left renal vein. The division of the left adrenal vein marks the lower most aspect of the dissection. Dissection below this plane, if necessary, should be done with the utmost caution to prevent an inadvertent injury to the renal vessels within the renal hilum. The dissection is continued from the superior pole of the kidney and the posterior musculature. Once the final attachments to the diaphragm are transected, the adrenal bed is examined for bleeding. The specimen is removed through the lateral most port in a bag. The steps of a left adrenalectomy are depicted in Fig. 13.3.

Retroperitoneal Laparoscopic

The posterior retroperitoneal approach is a valuable approach in patients requiring bilateral adrenalectomy, and in patients who have previously undergone abdominal surgery, who may have significant intra-abdominal adhesions. Once the patient has received adequate general anesthesia, and the appropriate intravenous and intraarterial access has been completed, the patient is placed in a prone jackknife position. The hips and knees are flexed at 90° and all pressure points are padded. The placement of cushions to support the hips and lower chest allows the anterior abdominal wall to hang in between. The tip of the 12th rib is palpated or visualized using an ultrasound, and a 1.5 cm transverse incision is made below the tip of the rib. This incision is extended into and through the posterior musculature and fascia to enter the retroperitoneum. A working space is created by blunt digital separation of the posterior abdominal wall fascia and the retroperitoneal fat. Under finger guidance, a 5 mm lateral trocar is inserted 4–5 cm from the initial incision, beneath the 11th rib. Next, a 10 mm trocar is inserted medially to the initial incision, 2–3 cm below the 12th rib, under finger guidance. A 10 mm balloon blunt trocar is inserted into the initial incision and secured, followed by insufflation with carbon dioxide (20–25 mmHg). The retroperitoneal fat and the adrenal gland are dissected free from the diaphragm superiorly and the superior pole of the kidney is retracted. The adrenal gland is mobilized both medially and inferiorly. For the right adrenal gland, the gland is dissected free from the inferior vena cava with identification of the right adrenal vein. For the left adrenal gland, the gland is dissected medially to identify the left adrenal vein. The adrenal vein is clipped and divided. The remaining attachments are dissected free laterally and superiorly. The dissection bed is checked for hemostasis and the specimen is removed.

Fig. 13.2 Sequential steps in laparoscopic right adrenalectomy. (a) The right triangular ligament is divided along the dotted line. (b) Dissection is continued top-down with identification of the right adrenal vein and/or accessory veins. (c) Right adrenal veins are clipped and transected. Dissection continues along the superior pole of the right kidney and posterior retroperitoneal attachments. D duodenum, IVC inferior vena cava, K kidney, L liver

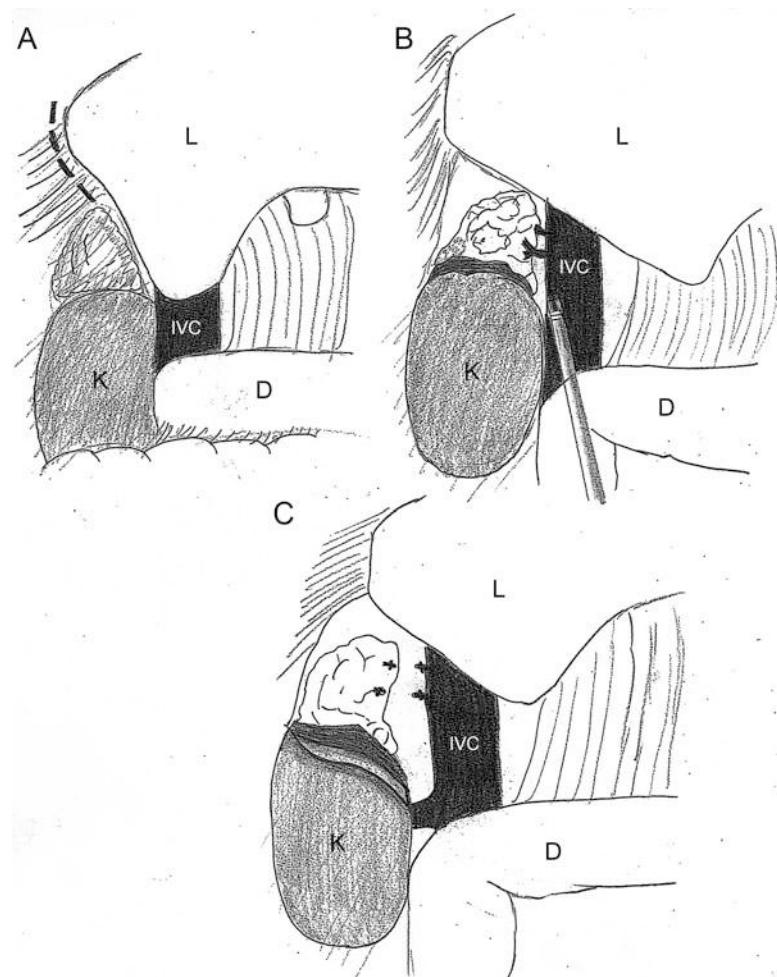
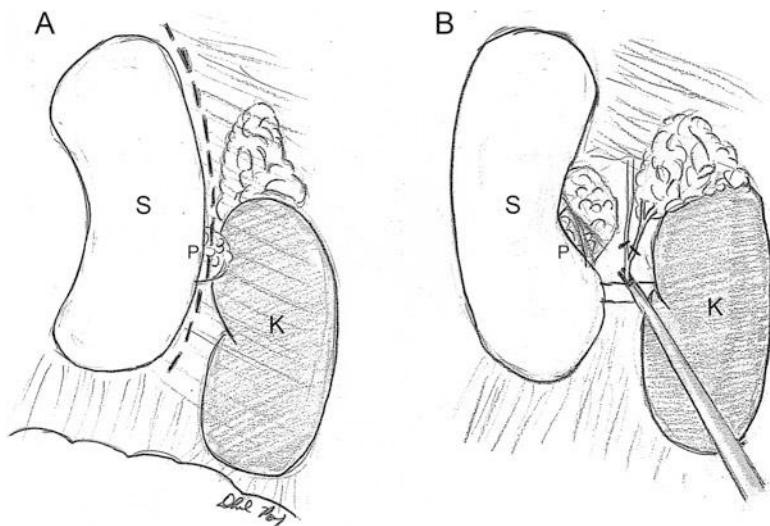


Fig. 13.3 Sequential steps in laparoscopic left adrenalectomy. (a) The peritoneum is divided along the dotted line, 1 cm lateral to the spleen. (b) Dissection is continued top-down with identification of the inferior phrenic vein and left adrenal vein. The veins are clipped and transected. Dissection continues along the superior pole of the left kidney and posterior retroperitoneal attachments. K kidney, P pancreas, S spleen



Laparoscopic Cortex-Sparing Adrenalectomy

In patients with a history of multiple endocrine neoplasia 2 (MEN2) and von Hippel Lindau (VHL) and bilateral pheochromocytomas, there is an indication to perform cortex-sparing adrenalectomy to avoid the risk of Addisonian crisis with long-term steroid replacement. Furthermore, these patients have a high risk of developing a contralateral pheochromocytoma, which would require an adrenalectomy. Laparoscopic cortex-sparing adrenalectomy is performed similarly to an adrenalectomy in terms of port placement and mobilization. Intraoperative ultrasound is utilized to help identify smaller tumors and the margins of resection. The adrenal gland parenchyma can be transected using electrocautery, and/or Harmonic® and LigaSure® scissors. The remnant gland is left in situ and not dissected. Dissection of the remnant gland may result in inadequate blood flow and should be avoided. This is especially true if the adrenal vein cannot be spared.

Open Adrenalectomy

In patients who may have malignant tumors, an open approach is the current optimal standard. Patients that have a large tumor, presence of local invasion, or presentation with locoregional recurrence, likely have a malignant tumor. A subcostal incision is performed two fingerbreadths below the costal margin. In patients with locally invasive tumors, a thoracoabdominal incision may be necessary for adequate vascular control. A retroperitoneal lymphadenectomy is routinely performed in patients with a possible malignant tumor. For an open right adrenalectomy, an extended Kocher maneuver is essential for adequate exposure and to allow for vascular control of the infrahepatic inferior vena cava (IVC). The triangular ligament should be divided, and the right lobe of the liver should be mobilized medially. If further exposure is necessary, the liver can be completely mobilized by releasing the falciform, coronary, and triangular ligaments. Small veins from the inferior vena cava to the caudate

lobe of the liver can be ligated and divided as needed to help with mobilization. The porta hepatis is dissected and encircled with a Rommel tourniquet in case a Pringle maneuver becomes necessary. Vascular control of the suprahepatic vena cava must be completed prior to dissection of the tumor from the vena cava or right adrenal vein. Once infrahepatic IVC, suprahepatic IVC, and porta hepatis vascular control have been obtained, in patients with a tumor thrombus, a venotomy may be carried out to extract the tumor thrombus. In cases where a prolonged occlusion of the IVC is anticipated or extensive intrahepatic tumor thrombus extension up to the right atrium, the operation should be performed on veno-venous bypass or cardiopulmonary bypass. Transesophageal echocardiography should be used to define the level of thrombus extension intraoperatively. Malignant tumors may have invasion into surrounding organs, and therefore, adjacent organs may need to be resected including the right kidney and right lobe of the liver.

For an open left adrenalectomy, there may be local invasion that may require resection of the tail of the pancreas and spleen. The gastrocolic ligament is initially divided and the pancreas is mobilized, in anticipation of an *en bloc* resection. If not, the left adrenal gland is exposed by medial rotation of the spleen, pancreas, and the left colon. If there is local invasion, vascular control of the renal vessels may be required. If the tumor invades the diaphragm, a cuff of diaphragm may be necessary to resect as well, in patients with locally invasive tumors. The diaphragm can be resected and closed either primarily or with a Gore-Tex patch graft. An angled chest tube is left above the diaphragm if the diaphragm is resected.

Extent of Resections

In patients with ACTH independent Cushing's syndrome and a unilateral cortisol producing adenoma, the current standard of treatment is laparoscopic total adrenalectomy. In patients with primary bilateral macronodular hyperplasia, the current standard of treatment is bilateral adrenalectomy. However, given the morbidity of

lifelong steroid therapy, Debillon and colleagues retrospectively assessed the utility of unilateral adrenalectomy for patients with Cushing's syndrome due to primary bilateral macronodular adrenal hyperplasia. Fifteen patients underwent unilateral adrenalectomy of the larger gland and were found to have normal or low urinary free cortisol postoperatively. In addition, metabolic complications such as hypertension, obesity, and diabetes had improved in the cohort. Two patients did experience a recurrence, where one was treated with mitotane, and the other patient underwent surgery. The rates of recurrence are low and the authors conclude that a unilateral adrenalectomy may be an option for patients with primary bilateral macronodular hyperplasia [7]. Although this study highlights a possible change in management, the current standard of care is bilateral adrenalectomy for patients with primary bilateral macronodular hyperplasia and Cushing's syndrome.

The extent of adrenalectomy for patients with Conn's syndrome has been debated in the literature as well. Ishidoya and colleagues compared patients with unilateral aldosterone producing adenoma who underwent laparoscopic total adrenalectomy ($n=63$) and those who underwent laparoscopic partial adrenalectomy ($n=29$). All of the patients who underwent laparoscopic total adrenalectomy showed improvement with hypertension. Two out of the 29 patients who underwent partial adrenalectomy continued to have hypertension without any improvement, representing a possible treatment failure [8]. Weisbrod and colleagues examined the adrenal histologic finding of patients who underwent adrenalectomy after lateralization by adrenal venous sampling and found that 15 % of patients had both an adenoma and hyperplasia. If these patients had undergone a partial adrenalectomy, these patients would likely not have been cured or had improvement with their hypertension. Sixteen percent of patients had adrenal hyperplasia and no adenomas. Thus, a total of 31 % of patients had adrenal hyperplasia with and without an adenoma that may account for their primary hyperaldosteronism [9]. A partial adrenalectomy in these patients would not have been beneficial. Given the possi-

bility of high failure rate of partial adrenalectomy, a total adrenalectomy continues to be the gold standard in the surgical treatment of unilateral aldosterone producing adenoma.

The goal of resection in patients with adrenocortical carcinoma is an R0 resection. In patients with Stage I adrenocortical carcinoma, an adrenalectomy with wide margins including the surrounding retroperitoneal fat and lymphatics may achieve R0 resection. In patients with Stage II and III adrenocortical carcinoma, complete removal of the tumor may require *en bloc* resection of the ipsilateral kidney, liver, spleen, pancreas, stomach, colon, or a portion of the inferior vena cava.

In patients with pheochromocytoma, the extent of resection and the possibility of cortical sparing surgery are based on three assumptions: (1) low risk of malignancy; (2) a low to moderate risk of recurrence that can be monitored, diagnosed, and cured; and (3) high likelihood of maintaining normal adrenal cortical function [10]. Patients with sporadic pheochromocytomas should undergo a total adrenalectomy. Patients with hereditary syndromes such as MEN 2A and VHL should be offered a partial adrenalectomy, given the high likelihood of recurrent contralateral pheochromocytoma and the morbidity of steroid dependence as detailed in the next section.

Outcome

The outcome for patients with Cushing's syndrome due to an adrenocortical adenoma is excellent. Many of these patients require steroid supplementation until the hypothalamus-pituitary-adrenal (HPA) axis recovers completely. Välimäki and colleagues reported long-term follow-up of fourteen patients who had undergone surgery for Cushing's syndrome. None of the patients showed relapse assessed by clinical features of Cushing's syndrome. Five of the fourteen patients, however, did not have their metabolic complications ameliorated, indicating that there may be other underlying etiologies. Postoperatively, thirteen of the fourteen of the patients were able to be weaned from their steroid replacement (mean 11.8

months, range 3 to 28 months) [11]. A systematic review analyzing the postoperative recovery of the HPA axis by Di Dalmazi and colleagues showed that the prevalence of adrenal insufficiency was 99.7% in patients with Cushing's syndrome who underwent adrenalectomy, as expected. Patients with subclinical hypercortisolism had a rate of adrenal insufficiency varying from 51.4 to 91.3% depending on the biochemical definition of subclinical hypercortisolism. The mean time to HPA axis recovery for patients with Cushing's syndrome was 11.2 months compared to 6.5 months for patients with subclinical hypercortisolism [12].

Symptom resolution of Cushing's syndrome after adrenalectomy has been examined by surgeons to identify the rate and time to resolution. Sippel and colleagues assessed symptom resolution and found that the majority of patients had their physical changes resolved within a mean of seven to nine months. However, symptom resolution could take up to 4 years. Quality of life improved in nearly 80% of patients. Medical comorbidities such as hypertension and diabetes also improved postoperatively. Forty-one percent of patients had diabetes preoperatively, and of those, 79% were cured postoperatively. Seventy-eight percent of patients had hypertension preoperatively, and of those, 67% had either improvement or cure postoperatively [13].

Although there is retrospective data indicating operative treatment in patients with subclinical Cushing's as beneficial, the optimal therapeutic paradigm has yet to be defined with quality evidence [14, 15]. Patients with subclinical hypercortisolism have biochemical evidence of hypercortisolism without overt clinical signs and symptoms. Retrospective analyses of patients who have undergone adrenalectomy have shown clinical benefit to patients with subclinical hypercortisolism. An earlier study with a small cohort of nine patients showed that six out of the eight patients with preoperative hypertension had improvement of their blood pressure postoperatively. A reduction of pharmacotherapy due to improved glycemic control was also shown in two out of three patients with preoperative diabetes. Nearly all of the patients had a reduction in

their weight postoperatively [16]. In another retrospective analysis of patients with subclinical hypercortisolism who either opted for adrenalectomy versus observation demonstrated significant benefit in the adrenalectomy group. In the study, 20 patients underwent operative intervention and 15 patients opted for conservative management. In the patients who had an operation, 53% of patients had an improvement in blood pressure and 50% had improved glycemic control, whereas there was no improvement in the control group [17]. Chiodini et. al. evaluated 41 patients who were diagnosed with subclinical hypercortisolism and either underwent adrenalectomy ($n=25$) or refused surgery ($n=16$). After an 18-month follow-up, a comparison of the two groups showed the adrenalectomy group to have a statistically significant improvement in weight (32 vs. 12.5%), blood pressure (56 vs. 0%), and fasting glucose levels (48 vs. 0%). The blood pressure (50 vs. 0%), fasting glucose levels (37.5 vs. 0%), and LDL levels (50 vs. 20%) worsened in patients who did not have adrenalectomy [18].

In one of the largest studies to date, a group in Italy prospectively analyzed the outcomes of 45 patients with subclinical hypercortisolism over a 15-year period. Twenty-three patients underwent adrenalectomy and 22 patients were observed. The prevalence of metabolic complications was similar between the two groups: diabetes (34.8% vs. 27.3%), hypertension (78.3% vs. 68.2%), hypercholesterolemia (34.8% vs. 31.8%), obesity (26.1% vs. 27.3%), and osteoporosis (21.7% vs. 27.3%). The analysis of the long-term outcome of the surgical group revealed a statistically significant improvement and/or normalization in 12 out of 18 patients with hypertension. Although statistically not significant, diabetes normalized and/or improved in five out of eight patients, hypercholesterolemia normalized in three out of eight patients, and the body mass index normalized in three out of six patients. Unfortunately, the authors did not do a data comparison between the medically managed group and the operative group [15]. Although these studies show resolution of metabolic complications, the studies suffer from small sample size, retrospective nature, lack of randomization, and lack of uniform medical management.

Currently, many surgeons will offer adrenalectomy to patients with subclinical Cushing's based on retrospective data.

The outcome of patients with aldosterone-producing adenomas who benefit from adrenalectomy has been studied extensively. Sawka and colleagues examined a large cohort of 97 patients with aldosterone-producing adenomas after adrenalectomy. Hypertension resolved in 33% of patients and hypertension improved (defined as a decrease in blood pressure or decrease in antihypertensive medications) in 98% of patients. Factors that were significantly associated with resolution of hypertension included a lack of family history (odds ratio of 10.9) and the preoperative use of two or fewer antihypertensive agents (odds ratio of 4.7) [19]. Zarnegar and colleagues assessed whether these and other preoperative factors may predict the resolution of hypertension in patients with aldosterone-producing adenomas. An easy to use aldosterone resolution score (ARS) was created and is presented in Table 13.3. The ARS consists of four predictors: 2 or fewer antihypertensive medications, body mass index $\leq 25 \text{ kg/m}^2$, duration of hypertension ≤ 6 years, and female sex. The likelihood of resolution is defined by the ARS score was 27% for those with a low score (0–1), 46% for those with a medium score (2–3), and 75% for those with a high score (4–5). These findings were validated in an independent set of patients [20].

Patients with aldosterone-producing adenomas who have undergone adrenalectomy have been noted to have an improvement in long-term cardiac morbidity. Catena and colleagues compared patients with primary hyperaldosteronism and patients with essential hypertension. The two groups were age and gender matched. Patients with primary hyperaldosteronism were treated with either adrenalectomy for aldosterone-producing adenomas or medical therapy with spironolactone for those with idiopathic bilateral primary hyperaldosteronism. Patients with primary hyperaldosteronism were more likely to report a history of cardiovascular events prior to treatment (35% vs. 11%, odds ratio 4.61) compared to those with essential hypertension. Patients were followed prospectively for any

Table 13.3 Aldosterone resolution score

Predictor	Points	
	Present	Absent
No. antihypertensive medications ≤ 2	2	0
Body mass index ≤ 25	1	0
Years of hypertension ≤ 6	1	0
Female	1	0
Total	5	0

Adapted from Zarnegar et al. [20], with permission

cardiac or vascular event such as myocardial infarction, stroke, revascularization procedures, and sustained arrhythmias. Nineteen percent of patients with primary hyperaldosteronism had a cardiac or vascular event compared to 18% of patients with essential hypertension (hazard ratio 0.93) [21]. Thus, even if patients with primary hyperaldosteronism do not have complete resolution of their hypertension, adrenalectomy may be beneficial to reducing cardiac morbidity.

In addition to improved blood pressure control and reduction in cardiac morbidity, many of these patients have an improvement in quality of life. The improvement of quality of life in these patients with primary hyperaldosteronism was assessed prospectively in a pilot study by the SF-36 health questionnaire. Twenty-two patients were assessed preoperatively, 3 months postoperatively, and 6 months postoperatively. These scores were then compared to general population SF-36 scores. There was a significant improvement in physical functioning, role limitations due to physical health problems, general health perceptions, role limitations due to emotional problems, mental health, and vitality at each time point compared to preoperative baseline [22]. Thus, the benefit of adrenalectomy in these patients goes beyond blood pressure control and reduction in cardiac morbidity.

Adrenal resection continues to represent the best treatment option for patients with adrenocortical carcinoma. One of the most important factors that influence the overall survival of patients undergoing adrenalectomy for ACC is an R0 resection. Margonis and colleagues in a multi-institutional study retrospectively compared patients who underwent surgery for adrenocortical

carcinoma and had either an R0 or R1 resection. On multivariate analysis, the 5-year overall survival was 96.3 months compared to only 25.1 months for those who underwent an R1 resection. Nearly 65% of patients with an R0 resection were alive at 5 years compared to only 33.8% of patients with an R1 resection [23]. This study emphasizes the importance of excellent surgical technique and preoperative planning to achieve an R0 resection to ensure the best possible outcome for patients with ACC. In a large single institution retrospective study, 330 patients with adrenocortical carcinoma were found to have a poor prognosis despite surgical therapy. Surgical resection in that cohort was performed on 275 patients with adrenocortical carcinoma and the median local-recurrence free time was slightly over one year. Positive surgical margins and advanced disease stage were associated with local recurrence. Median overall survival time for all patients was 3.21 years and became progressively shorter based on higher stage. In a multivariate analysis, older age, functioning tumors, and higher disease stage were associated with poor survival [24]. The outcome of patients with adrenocortical carcinoma continues to be poor.

The oncologic impact of lymphadenectomy in patients with adrenocortical carcinoma has been examined by Reibetanz and colleagues. A retrospective study examined the results of 283 patients who underwent surgery. Forty-seven (16.6%) of patients were treated with lymphadenectomy compared to 236 patients (83.4%) who were treated with surgery without lymphadenectomy. On multivariate analysis, lymph node status was associated with reduced risk for tumor recurrence and for disease-related death in patients who had a lymphadenectomy [25]. Although these results may indicate the impact of lymphadenectomy on the oncologic outcome of patients with adrenocortical carcinoma, the limitations to the study including a retrospective study design, and low rate of lymph node dissection.

Patients with pheochromocytoma must have lifelong follow-up after surgical resection. Khorram-Manesh and colleagues assessed the outcome after adrenalectomy for 121 patients

with an average follow-up time of 15 ± 6 years. Eight of the 121 patients with sporadic tumors on follow-up presented with recurrence after a mean of 8.5 ± 6 years after surgical resection with four of the eight patients dying from their disease. During the entire period of observation, 42 patients died compared to 23.6 expected deaths in the general Swedish population. The rate of hypertension was also examined with 85% of patients having hypertension at the time of diagnosis. At 1 year after surgery, there was improvement, but half the patients were still hypertensive. Although the rate of malignancy and death from malignancy was not high, patients with pheochromocytomas in the long term had an increased risk of death [26].

The outcome of patients with hereditary pheochromocytomas who have a germline mutation in the succinate dehydrogenase subunit B (SDHB) differs from those patients with sporadic pheochromocytomas. Patients with *SDHB* mutation are at higher risk for metastatic disease and disease-specific mortality. A retrospective study by Schovanek and colleagues showed that patients with a pheochromocytoma or paraganglioma greater than 4.5 cm were at a significantly higher risk of developing metastatic disease. In addition, patients with tumors greater than 5.5 cm had an overall worse survival compared to smaller tumors. The age of initial diagnosis was also found to be an independent predictor of survival [27]. Thus, patients with pheochromocytoma require lifelong biochemical and imaging surveillance.

Patients with hereditary syndromes present a challenge in management and approach. Patients with MEN 2A and VHL are predisposed to pheochromocytomas. Since these patients are predisposed to pheochromocytomas and have a high likelihood of recurrent pheochromocytomas, many surgeons have advocated a cortical sparing adrenalectomy approach to these patients. Benhammou and colleagues reported the rate of local tumor recurrence, the need for subsequent intervention, and the oncologic efficacy in the long-term outcome of patients with VHL. Thirty-six patients underwent successful partial adrenalectomies either through a laparoscopic or open approach.

Eleven percent of patients developed local recurrences and 11% of patients subsequently required partial adrenalectomy on the contralateral adrenal gland. No patients developed metastatic pheochromocytoma with at least 5 years of follow-up (median follow-up 9.25 years). Long-term steroid therapy was required in 11% of patients who either had a contralateral total or partial adrenalectomy [28]. The authors concluded that partial adrenalectomy is a reasonable option in patients with VHL-associated pheochromocytomas.

In one of the largest studies examining the surgical approach and treatment to patients with MEN 2A, Castinetti and colleagues compiled a database of 1210 patients with MEN 2A from 30 different academic medical centers in Europe, America, China, and India. Of these patients, 563 presented with pheochromocytomas with 44% with bilateral and 56% with unilateral pheochromocytomas. Of the patients with unilateral pheochromocytomas, 30% would subsequently develop a contralateral pheochromocytoma during follow-up. In the 552 patients who had surgery, 79% underwent either unilateral or bilateral adrenalectomy compared to 21% of patients who underwent cortical sparing adrenalectomy. Three percent of patients with cortical sparing adrenalectomy had a recurrence compared to 2.5% of patients with adrenalectomy. The mean follow-up time was 13 years and there was no significant difference in recurrence between these two groups. Steroid dependency was 42.7% of patients with bilateral pheochromocytomas who underwent cortical sparing adrenalectomies compared to 100% of patients with bilateral pheochromocytomas who underwent bilateral adrenalectomies. Given the risk of Addisonian crisis, the authors conclude that patients with MEN 2A should undergo cortical sparing adrenalectomy [29].

In conclusion, the evaluation of an adrenal mass requires a stepwise approach to determine whether any type of surgical or medical intervention is necessary. Laparoscopic adrenalectomy has become the standard approach except in patients with adrenocortical carcinoma or malignant pheochromocytoma. The extent of resection is dictated by the underlying disease process, genetic back-

ground, and the technical success for a superior oncologic outcome. Although the outcome for patients with benign adrenal disease is excellent, the outcome for adrenocortical carcinoma continues to be poor without a R0 resection.

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Therapies for Locally Advanced and Metastatic Adrenocortical Carcinoma

Sarika N. Rao and Mouhammed Amir Habra

Standard Therapy

Overview

Adrenocortical carcinoma (ACC) is a rare cancer, with an estimated incidence of 0.7–2 cases per million per year [1–3]. Overall, prognosis is poor, especially in those with advanced disease, either stage III (primary tumor of any size with lymph node involvement or invasion into local soft tissue or other organs, or tumor thrombus in renal vein or inferior vena cava) or stage IV (distant metastasis). It is estimated that 16–34 % of ACC patients have stage III disease at the time of diagnosis, while stage IV disease is seen in 21–36 % of patients [4–7]. The 5-year overall survival rate is around 50 % among patients with stage III ACC and less than 15 % among those who present with metastatic disease [5, 6, 8]. The management of advanced ACC consists of surgical resection for localized disease, with perioperative control of hormonal excess, and occasional use of neoadjuvant antineoplastic

agents. Local and distant recurrence rates after primary surgical resection remain high, and recurrences are estimated to occur in two-thirds of patients after seemingly complete resection; thus, adjuvant therapy is often used to prolong recurrence-free survival [9]. Current medical strategies are of suboptimal efficacy, however, primarily consisting of a combination of mitotane and traditional chemotherapy. Radiotherapy is also indicated in select cases but is mostly viewed as a palliative measure in patients with advanced ACC.

In the first half of this chapter, we will review the current standard medical therapies for advanced ACC, their efficacy and limitations, and specific indications for their use. In the second half, we will discuss newer trends in cancer medicine with the application of targeted molecular therapy and its impact on ACC.

Hormonal Management

Hormonal excess is seen in about 40–70 % of ACCs [4, 10, 11]. A wide range of complex and challenging metabolic, musculoskeletal, infectious, and cardiovascular derangements can be seen in hormonally active ACCs and can further complicate the management of this already aggressive malignancy. In a study of 524 ACC patients who underwent surgical resection, 275 (52.5 %) had hormonally active disease. Cortisol

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overproduction was most prevalent, seen in 150 (28.6%) of these patients, followed by androgen excess in 58 patients (11.1%), estrogen excess in nine patients (1.7%), mineralocorticoid excess in seven patients (1.3%), and multihormonal secretion (usually cortisol and excess steroids) in nearly 10 % of the patients [10]. This distribution of hormonal overproduction in ACC is relatively consistent with other studies [4, 5].

Due to the aggressive nature of ACC, therapeutic management focuses on reducing tumor burden. Surgical cytoreduction, when feasible, can be justified in select patients with advanced disease/metastatic ACC, but this strategy should be considered only by multidisciplinary teams with known experience in ACC management [11, 12]. For hormonally active unresectable ACCs and unresectable recurrences or in the pre-operative setting, medical therapy is required for hormonal control aiming to reduce the morbidity of hormonal excess. Excesses of cortisol and other hormones in ACC are most commonly controlled through the use of mitotane (which has the combined advantage of adrenolysis and steroidogenic inhibition), steroidogenesis enzyme blockers (ketoconazole, metyrapone, or rarely etomidate), glucocorticosteroid blocker (mifepristone), or mineralocorticoid blockers (spironolactone or eplerenone) [13]. Patients often require multiple medications, with a high potential for drug–drug interactions. As a result, very close clinical assessment and laboratory monitoring are needed to adjust the treatment plans based on the patients' response to therapy as well as the side-effect profile of the drugs used. Localized therapy for select patients with dominant hepatic metastases, such as hepatic chemoembolization, may decrease hormonal secretion, but the decision to perform such intervention should be individualized [14].

Surgery for Advanced/Metastatic ACC

Recurrent or metastatic ACC carries a grim prognosis, and surgery often has a limited role in management. Nevertheless, surgery should be considered for recurrent disease if it is local and surgically accessible, with the ability to achieve

negative resection margins [15, 16]. In addition, cytoreductive surgery should also be considered in cases where resection of the bulk of disease may decrease the morbidity associated with cortisol excess. Resection of metastatic sites can also be an appealing choice for select patients who usually have a more indolent disease course and have few sites of disease in a limited number of organs (usually liver and/or lungs).

In a retrospective review of 253 ACC patients (57 [22.5 %] with stage III and 54 [21.3 %] with stage IV disease), 182 patients (71.9 %) underwent curative surgery, and 105 patients (41.5 %) had extensive surgery. Adjuvant or neoadjuvant mitotane was used in 65 of the 111 stage III and IV patients (58.6%). The 5-year rate of overall survival was 24 % in stage III and 0 % in stage IV patients [6].

In another study assessing surgical outcomes in seven patients with advanced ACC, mitotane was not used adjuvantly. Tumors from five patients with stage III and two patients with stage IV disease were resected en bloc. Combined resection of the liver and inferior vena cava was performed in six patients, and two patients underwent kidney resection. Though mitotane was not used immediately postoperatively, five patients did receive mitotane at the time of tumor recurrence. The 3-year disease-free and overall survival rates for patients with stage III disease were 20 and 40 %, with a median follow-up time of 32 months. The estimated 3-year disease-free survival rate was 14.3 % and the median disease-free survival time was 18.6 months for all 7 stage III and IV patients [17].

In a series of 113 patients with ACC, of whom 107 had surgery, the median overall survival time for those with stage III or IV disease ($N=56$) was 15 months, and the 5-year overall survival rate was 10 %. Complete repeat resection was performed in 62 patients. Of the complete resections, 43 (69 %) were for distant metastases, 14 (23 %) were for local recurrences, and 5 (8 %) were for both; median survival in this group was 74 months, with a 5-year overall survival rate of 57 %. Among those with incomplete resections, however, the 5-year overall survival rate was 0 % [18]. In another series from the National Cancer Institute

of 57 patients who underwent either metastasectomy or repeat resection for metastatic ACC, 19 were treated for local recurrence and the remaining 38 had distant disease. In total, 116 metastasectomies were performed (at first recurrence or in subsequent surgeries), primarily for lesions in the lung/thoracic cavity (48 procedures) or liver (23 resections after the first metastasectomy, 22 patients (39 %) were classified as having no evidence of disease (NED) and 28 patients had two or more surgeries for recurrent disease. Disease-free interval (DFI) ranged from 2.8 months to greater than 12 years (median 4.1 years) and was determined for those 22 patients who were considered NED after first metastasectomy [19]. Extrapolating these results to clinical practice suggests the disease-free interval should be included when evaluating patients for repeat resections, i.e., the longer that interval, the longer the overall survival time. Both of these studies highlight the potential role of surgical intervention in select ACC patients with recurrent disease.

As earlier, resection may be considered in cases where isolated sites of metastatic disease can be easily resectable as it improves overall survival [18, 20–23]. In a study of 27 patients from Mayo Clinic and MD Anderson Cancer Center undergoing metastasectomy, sites included the lung (19), liver (11), and brain (1). Complete resection (R0) was achieved in 11 patients, who then had a median overall survival time of 2.35 years. The location of the metastasis was not associated with the length of overall survival after surgery [15].

In a study of 28 patients who underwent resection for liver metastasis from ACC (11 with synchronous metastasis), all 28 patients developed recurrent disease. The median disease-free and overall survival times after hepatectomy were 7 and 31.5 months, respectively, with a 5-year survival rate of 39 % [23]. Similarly, two retrospective studies from Germany and the U.S. National Institutes of Health (NIH) of 50 patients combined who underwent pulmonary metastasectomies found the 5-year overall rate of survival (calculated from the time of the first pulmonary surgery) to range from 24.5 to 41 %, with median overall survival time of 40–50.2 months [22, 24].

A median ipsilateral recurrence-free survival time of 6 months was reported from the NIH study [22]. All patients had recurrence of their pulmonary metastasis. Though rarely curative, resection of liver and lung metastases was associated with long-term survival.

Radiotherapy

Few patients with ACC receive radiotherapy. The use of radiotherapy for local control is suggested based on retrospectively collected data from limited number of patients because of the rarity of ACC and the infrequent use of radiotherapy. It has been suggested that radiotherapy can be more effective in the context of mitotane therapy based on cell culture data. While there are no published prospective data about its role, radiation treatment is often used as a palliative tool either alone or in combination with systemic therapy [25].

Mitotane Monotherapy

Mitotane is the only U.S. Food and Drug Administration-approved drug for metastatic ACC, although it is often used as adjuvant therapy in patients with advanced ACC. Mitotane has both adrenolytic and steroidogenesis blockade activities. It is often indicated when surgery is incomplete or contraindicated and in patients with hormonally functioning tumors. There have been isolated case reports of patients with metastatic disease in whom mitotane monotherapy resulted in prolonged remission [26–28]. Published trials on mitotane monotherapy are generally retrospective, with partial response only seen in 10–30 % of patients [11, 29–32]. Two small prospective studies involving adjuvant mitotane use yielded similar response rates [33, 34]. The toxic effects of mitotane include adrenal insufficiency due to the agent's adrenolytic effect on the adrenal tissue and activation of CYP3A4 leading to increased hydrocortisone inactivation, necessitating concurrent administration of higher-than-average glucocorticoid replacement. During mitotane therapy, many clinicians favor

using hydrocortisone replacement over other glucocorticosteroids (dexamethasone or prednisone). While there are different ways to replace steroids, an acceptable method is to start hydrocortisone 10 mg twice daily upon the initiation of mitotane or shortly after and titrate the dose based on clinical assessment and laboratory tests. Other common side effects of mitotane include digestive effects (nausea, vomiting, or diarrhea) that can occur early during therapy and can be managed with dose reduction and the use of supportive therapy. Neurological systems (dizziness, somnolence, depression, and ataxia) are often associated with higher mitotane levels and can be reversed with dose reduction or drug discontinuation. Other less common side effects can include hepatotoxicity with elevated liver enzymes, skin rash, hyperlipidemia, painful gynecomastia, thyroid dysfunction, and rarely hemorrhagic cystitis [11, 12, 35, 36].

Mitotane is often initiated in low doses of 2–3 g daily and can be titrated based on tolerance and periodic monitoring of mitotane serum levels (oncological target 14–20 mg/L) [37]. In a prospective multicenter trial including 40 ACC patients who received either low-dose (with slower dose escalation from 1 to 3 g over a 2 week period) or high-dose (with faster dose escalation from 1.5 to 6 g over a 2 week period) mitotane, there were no statistically significant differences in the median maximum plasma mitotane levels or side effect profiles [38]. In general, patients starting mitotane are counseled about adverse effects, including the need for steroid replacement while on therapy. Adrenal insufficiency is often reversible after discontinuing mitotane, but it can take few months for cortisol production to normalize after mitotane cessation. During mitotane use, we usually measure serum mitotane levels every 3–4 weeks initially and less frequently thereafter. Other values monitored include complete blood count; thyroid, renal, and liver functions; electrolyte levels; and lipid profile. Other tests can be added, especially in cases of hormonally active ACC, to assess the disease's response to therapy and to adjust the dose of glucocorticosteroid replacement.

Systemic Chemotherapy

Systemic therapy is the treatment of choice in metastatic ACC, and both single agents and combination therapies have been used to treat advanced ACC, as summarized in Table 14.1.

Single-Agent Chemotherapy

Limited experience has been reported using single-agent chemotherapy in ACC. Some case reports have indicated that cisplatin is active against ACC; however, the effects were minimal and combination therapy was recommended [39, 40].

Doxorubicin was used as a single agent (60 mg/m^2 every 3 weeks) in 52 patients with advanced ACC that did not respond to mitotane, had a poorly differentiated histology, or was hormonally inactive. Response to doxorubicin was seen in 3 of 16 patients who did not have prior mitotane exposure (19 %), while there were no responses to doxorubicin in patients in whom mitotane failed [30]. Irinotecan, a topoisomerase I inhibitor, was given to as a single agent (250 mg/m^2 every 14 days) to 12 patients with advanced ACC. There were no objective tumor responses, and only three patients (25 %) had short-lived disease stabilization (range 1.5–4 months) [41].

Recently, trofosfamide, an orally administered alkylating agent that belongs to the cyclophosphamide family, has shown some efficacy in the treatment of sarcoma and some gynecologic cancers [42, 43], and overall it has been well tolerated. Hence, the potential for use of this drug in ACC was evaluated in a retrospective analysis of compassionate use of trofosfamide (150 mg daily) in 27 patients (13 patients received it as monotherapy and 14 in combination with mitotane). Only three patients had stable disease, the longest duration of which was 479 days. Median progression-free survival for the entire group was 84 days; median overall survival was 198 days. Though trofosfamide was overall well tolerated, its effectiveness as salvage therapy in ACC is very limited [44].

Table 14.1 Summary of systemic chemotherapies studied in adrenocortical carcinoma

Investigator	Drug(s) (dose)	No. of patients	Study design/ setting	No. of patients with response (type if reported)	Response duration
Decker et al. [30]	Doxorubicin (60 mg/m ² every 3 weeks)	16	Prospective, initial treatment	3	Not reported
Baudin et al. [41]	Irinotecan (250 mg/m ² every 2 weeks)	12	Prospective	3 (SD)	1.5–4 Months
Krois et al. [44]	Trofosfamide (150 mg daily)	27 (14 combined with mitotane)	Retrospective, salvage	3 (SD)	84 Days
Khan et al. [45]	Mitotane (1–4 g orally daily) + streptozotocin (1 g intravenously daily × 5 days, then 2 g IV every 3 weeks) (M-S)	40	Phase II	14	
Williamson et al. [33]	Cisplatin (50 mg/m ² on days 1 and 2) + etoposide (100 mg/m ² on days 1, 2, and 3; cycle every 21 days)	45 (9 patients previously received mitotane)	Phase II	5 (PR)	
Berruti et al. [50]	Mitotane (4 g/day) + etoposide (100 mg/m ² on days 5–7) + doxorubicin (20 mg/m ² on days 1 and 8) + cisplatin (40 mg/m ² on days 1 and 9) every 4 weeks (EDP-M)	72	Phase II	5 (CR) 30 (PR)	
Fassnacht et al. [51]	EDP-M (per Berruti protocol [50]) vs. mitotane + streptozotocin (M-S) (per Khan protocol [45])	304 (151 in EDP-M and 153 in M-S)	Phase III	EDP-M: – 2 (CR) – 33 (PR) – 53 (SD) M-S: – 1 (CR) – 13 (PR) – 34 (SD)	5 Months vs. 2.1 months
Sperone et al. [62]	Mitotane plus gemcitabine (800 mg/m ²) + capecitabine (1500 mg/day) or 5-fluorouracil (200 mg/m ² /day)	28	Phase II, salvage	1 (CR) 1 (PR) 11 (SD)	CR (20 months) PR (10)

SD stable disease, PR partial response, CR complete response

Combination Chemotherapy

Mitotane and Streptozotocin

In a small phase II trial including 40 patients with recurrent or metastatic ACC who received combination mitotane (1–4 g per day) and intravenous streptozotocin (1 g per day for 5 days and then 2 g every 3 weeks). Complete or partial

responses were reported in 36 % of subjects, with a 2-year overall survival rate of 70 % [45]. Neoadjuvant streptozotocin and mitotane were administered in a limited case series in which two of three patients with inoperable, advanced disease were later deemed to have developed operable lesions; response was also seen biochemically and at metastatic lesions [46].

Mitotane Plus Etoposide, Doxorubicin, and Cisplatin

In preclinical experiments, synergistic activity was observed with etoposide and cisplatin [47]. An early report of two ACC patients treated with the combination of etoposide and cisplatin suggested the efficacy of this combination [48]. A subsequent phase II trial of cisplatin (50 mg/m² on days 1 and 2) and etoposide (100 mg/m² on days 1, 2, and 3) given in a 21-day cycle enrolled 45 patients with advanced ACC who either had no prior therapy (36 patients) or had previously received mitotane (9 patients). Partial response was the best outcome achieved, seen in five patients, yielding an 11% response rate. In the mitotane-naïve group, 16 patients went on to receive adjuvant mitotane at disease progression, with partial response in two patients [33]. In ACC, the multidrug resistance gene (*MDR-1*) is highly expressed, and high levels of p-glycoprotein are expected [49]. The addition of mitotane interferes with p-glycoprotein and enhances drug accumulation and toxicity; therefore, mitotane with cytotoxic chemotherapy appears to be a rational combination. In a phase II study of 72 patients with metastatic or locally advanced ACC, mitotane (4 g per day) in combination with intravenous etoposide (100 mg/m² on days 5–7), doxorubicin (20 mg/m² on days 1 and 8), and cisplatin (40 mg/m² on days 1 and 9) (EDP-M) given every 4 weeks led to an objective response in 48.6% of patients (5 subjects with complete response and 30 with partial response) [50]. Subsequently, in the largest study and first phase III clinical trial in ACC patients, the First International Randomized trial in locally advanced and Metastatic Adrenocortical Carcinoma Treatment (FIRM-ACT), 304 patients with advanced ACC not amenable to radical surgery were randomized to mitotane with either EDP or streptozotocin. Response rates were significantly better in the patients receiving EDP-M than in the streptozotocin-mitotane group (23.2% vs. 9.2%), and median progression-free survival was also longer in the EDP-M group than in the streptozotocin-mitotane group (5.0 months vs. 2.1 months). The median overall survival time was not statistically different between the two groups (15 vs. 12 months), nor was the incidence of serious

adverse events [51]. This landmark study highlighted the need to uncover the molecular pathogenesis and mechanism of resistance in ACC and to explore more effective and less toxic regimens to treat ACC.

The suggested mechanisms in ACC resistance to chemotherapy include higher levels of p-glycoprotein, which have been associated with resistance to doxorubicin in particular [52]. Excision repair cross-complementary group 1 (ERCC1), a means by which tumor cells can offset the effects of platinum-based therapy, has been shown to be inversely proportional to the length of survival in patients with refractory ACC treated with cisplatin [53, 54]. Recently, a finding of cMET overexpression in response to cisplatin or mitotane exposure in an ACC cell line was proposed as another potential mechanism in the limited response of ACC patients to currently used chemotherapy [55].

While the combination of cytotoxic chemotherapy with mitotane has limited utility in long-term ACC management, this combination can be used as a bridge to definitive surgical resection in borderline resectable ACC (Fig. 14.1). In 15 patients whose ACC was retrospectively considered borderline resectable (based on imaging suggestive of a need for multiorgan resection, the presence of small-volume oligo-metastases, or the presence of unfavorable patient characteristics/poor performance status), systemic neoadjuvant therapy resulted in an objective response rate of 39% and 54% disease stability. The median disease-free survival time for this highly selected group was 28.0 months, compared to 13.0 months for 38 ACC patients who had surgery as their initial treatment. These findings highlight the importance of combining different modalities, including surgery and chemotherapy, to improve long-term outcomes in ACC; a prospective study is currently being developed to validate these earlier retrospective observations [56]. 18-fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) has been proposed as a useful tool to monitor and guide response to systemic therapy in ACC, with slight superiority over traditional CT. FDG PET/CT use changed clinical management in about 10% of cases

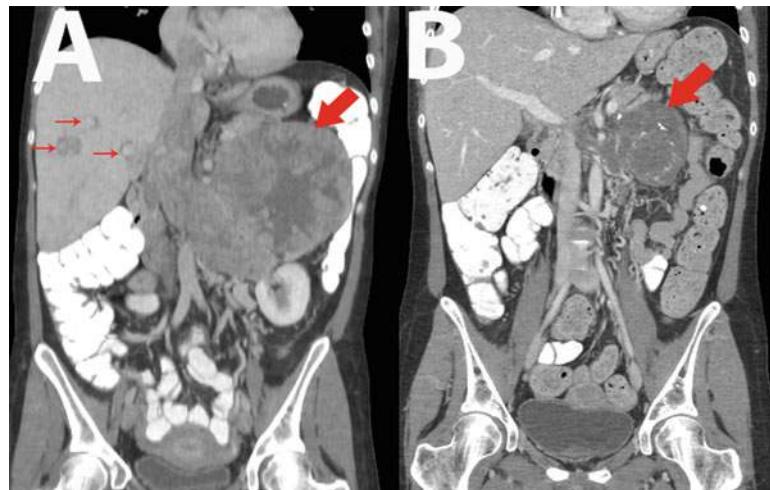


Fig. 14.1 Computed tomography images (coronal views) illustrating the response to neoadjuvant chemotherapy in a patient with adrenocortical carcinoma: (a) left-sided adrenocortical carcinoma (*thick red arrow*) extending through the left renal vein to the inferior vena cava and right atrium with multiple hepatic metastases (*thin red*

arrows). In (b) the response to therapy is demonstrated with remarkable reduction in primary tumor size (*thick red arrow*) and tumor vascular extension with resolution of liver metastases. This response facilitated successful and more limited surgical resection with curative intent

during restaging and had sensitivity and specificity of about 98.4 and 100%, respectively, during restaging [57, 58] (Fig. 14.2).

Mitotane Combination with Gemcitabine and Fluoropyrimidines

At the 2003 ACC consensus conference, gemcitabine was considered among the most promising agents [59], and the combination of it with a fluoropyrimidine, such as capecitabine or 5-fluorouracil, is effective in advanced pancreatic cancer [60] and modestly effective in resistant renal carcinoma [61]. A phase II study evaluated the combination of mitotane plus gemcitabine (800 mg/m^2) and capecitabine (1500 mg per day) or 5-fluorouracil (200 mg/m^2 per day) as salvage therapy in 28 patients (22 patients receiving capecitabine and 6 patients receiving 5-fluorouracil) whose disease had progressed after previously receiving mitotane plus one or two systemic chemotherapy lines. Ultimately, there was no difference between the fluoropyrimidines when combined with gemcitabine and mitotane. Complete response (lasting over 20 months) was observed in one patient with metastatic disease to liver and

bone, and another patient achieved a partial response (lasting 10 months); 11 patients showed disease stabilization, while the remaining 15 patients had disease progression. Among the 28 patients, median time to progression was 5.3 months [62].

Emerging Therapies

Overview

Considering the high rate of treatment failure and the significant clinical heterogeneity in ACC, significant effort was spent over the past two decades to understand the molecular features and cellular pathways involved in ACC pathogenesis. These efforts aimed to guide therapy and predict prognosis at an individual level [63]. The most commonly seen alterations in ACC include the overexpression of insulin-like growth factor 2 (IGF2), which leads to the activation of insulin-like growth factor receptor-1 (IGF-1R), and the activation of beta-catenin signaling (Fig. 14.3). Recent efforts to further our knowledge about ACC via whole-exome sequencing have identi-

Fig. 14.2 Coronal views from fluorodeoxyglucose (FDG)-positron emission tomography (PET). (a) The image shows a large FDG-avid left-sided adrenocortical carcinoma with vascular extension (thick red arrow) with liver metastases before therapy, and in (b), there is remarkable reduction in FDG uptake in the left adrenal mass (thick red arrow) and disappearance of the hepatic metastases in response to therapy

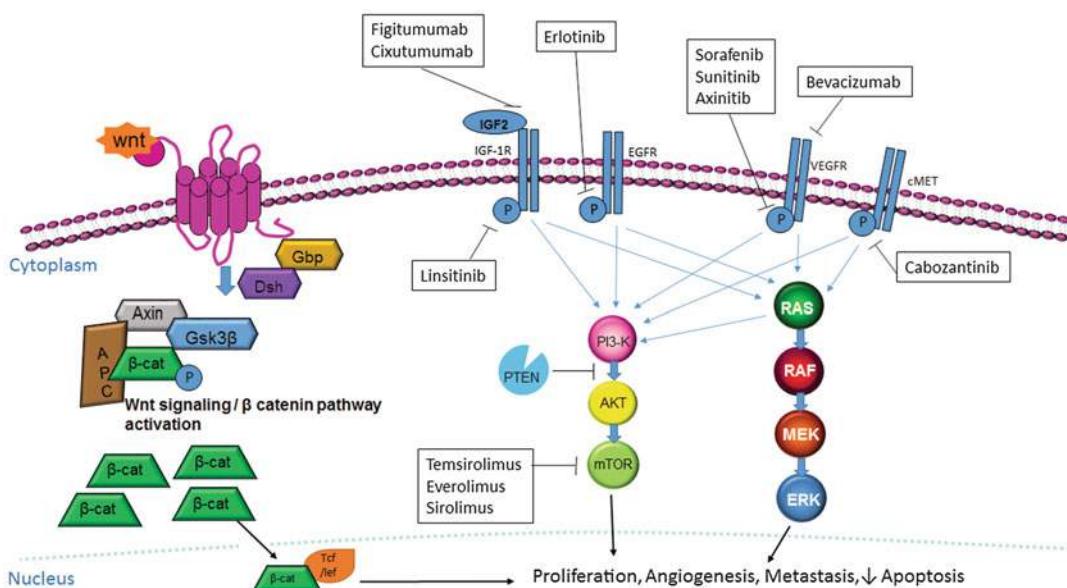
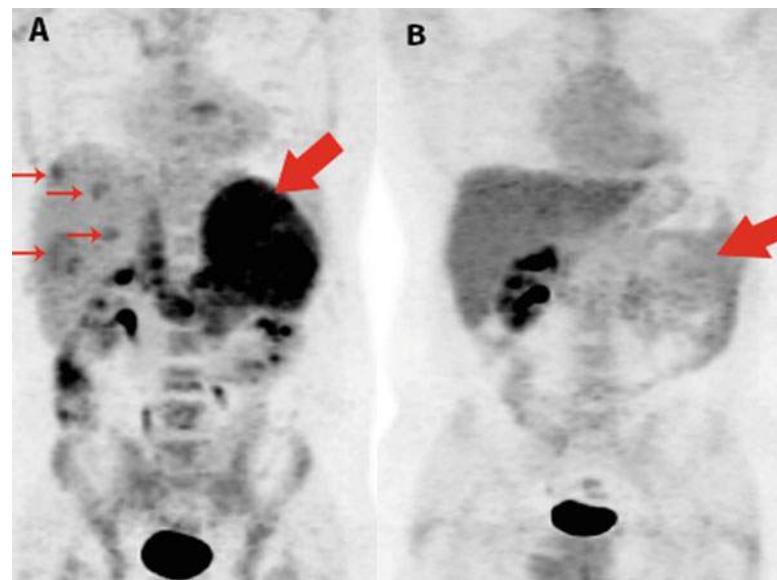


Fig. 14.3 Simplified illustration of intracellular signaling pathways in adrenocortical carcinoma showing select clinically available drugs to target these pathways

fied alterations in known driver genes (*CTNNB1*, *TP53*, *CDKN2A*, *RB1*, and *MEN1*) as well as in genes less commonly associated with ACC (*ZNFR3*, *DAXX*, *TERT*, and *MED12*) [64]. Comprehensive genomic profiling of 91 primary ACC cases also found many of the same driver genes (*TP53*, *ZNFR3*, and *CTNNB1*), along with some unique ones (*PRKARIA*, *CCNE1*, and

TERF2). Whole-genome doubling in ACC was a common feature and was associated with more aggressive behavior. The impact of these newly published data on clinical management of ACC is still unclear, as we discuss in this second half of Chap. 14, but these findings could lay the foundation for testing future classes of molecular targeted therapies in ACC (Table 14.2) [65].

Table 14.2 Summary of targeted therapies studied in adrenocortical carcinoma

Investigator	Drug(s) (dose)	Target	No. of patients	Study design	Best response (no. of patients)	Median PFS
Berruti et al. [79]	Sorafenib (400 mg twice daily) + paclitaxel (60 mg/m ² every week)	VEGFR	25	Phase II, salvage	No response	Not reported
Kroiss et al. [80]	Sunitinib (50 mg daily × 4 weeks)	VEGFR	38	Phase II	SD (5)	5.6–11.2 months
Wortmann et al. [83]	Bevacizumab (5 mg/kg IV every 21 days) + capecitabine (950 mg/m ²)	Anti-VEGF monoclonal antibody	10	Salvage	No response	Not reported
O'Sullivan et al. [84]	Axitinib (5 mg twice daily)	Specific VEGFR	13	Phase II	Reduced growth rate (4)	5.48 months OS; 13.7 months
Ganesan et al. [86]	Lenalidomide + temsirolimus	Antiangiogenic + mTOR inhibitor	3	Phase I (basket trial)	SD (2)	Not reported
Quinkler et al. [92]	Erlotinib (100 mg daily) + gemcitabine (800 mg/m ² IV q 2 weeks)	EGFR	10	salvage	PR (1)	8 Months
Haluska et al. [102]	Figitumumab (20 mg/kg on day 1 of each cycle)	Anti-IGF-1R monoclonal antibody	14	Phase I	SD (8)	6 Months
Naing et al. [108]	Cixutumumab (3–6 mg/kg every week) + temsirolimus (25–37.5 mg IV weekly)	Anti-IGF-1R monoclonal antibody + mTOR inhibitor	26	Phase I	SD (11)	>6 Months
Lerario et al. [103]	Cixutumumab (10 mg/kg every 2 weeks) + mitotane (2 g daily)	Anti-IGF-1R monoclonal antibody	20	Phase I	PR (1) SD (7)	2.66–48 weeks
Jones et al. [106]	Linsitinib (>300 mg)	IGF-1R and insulin inhibitor	66	Phase I PR (2) SD (27)	PR: 199–703 days SD: <24 weeks (not median)	PR: 199–703 days SD: <24 weeks (not median)
Fassnacht et al. [107]	Linsitinib (150 mg daily)	IGF-1R and insulin inhibitor	90	Phase III (vs. placebo)	No response	Not reported

PFS progression-free survival, SD stable disease, PR partial response, CR complete response, BID twice a day, OS overall survival

Antiangiogenic Therapy

Anti-VEGF Therapy

In general, malignant tumors are dependent on an adequate blood supply to support growth and invasion. Vascular-endothelial growth factor (VEGF) is the primary signal protein that binds to its tyrosine kinase receptor, inducing a signaling cascade causing endothelial proliferation and migration into sites of neovascularization [66]. Not surprisingly, its overexpression in cancer has been a target for treatment. Anti-VEGF agents have shown very encouraging results in cancer therapies, including those for advanced colorectal [67], renal [68], and lung [69] cancers, and this has led to an interest in targeting VEGF signaling in ACC [70].

VEGF expression has been shown to be higher in ACC than nonmalignant adrenal tumors [71–73]. Additionally, VEGF expression, particularly the subtype VEGF2, correlates with poorer outcomes in ACC [74]. Hence, blocking this signal may be crucial in treatment. In xenograft models of ACC, the multiple kinase inhibitor sorafenib, which has anti-VEGF activity, in combination with the mTOR inhibitor everolimus showed evidence of growth inhibition, tumor mass reduction, and increased apoptosis at both primary and metastatic sites [75].

Isolated case reports have described partial responses to sorafenib and a similar agent, sunitinib, in metastatic ACC [76–78]. In a phase II study of 25 patients, sorafenib combined with paclitaxel, though well tolerated, was discontinued early due to tumor progression in the first nine patients [79]. In another phase II trial, single-agent sunitinib was given in 6-week cycles (50 mg sunitinib daily for 4 weeks, followed by 2 weeks off) to 38 patients with advanced, nonsurgical ACC patients after previously that had not responded to mitotane and one to three chemotherapeutic agents. Over half the patients were still on concomitant mitotane. Only five patients had stable disease, with progression-free survival times ranging from 5.6 to 11.2 months and overall survival times ranging between 14.0 and 35.5 months. Of those five patients, only 1 was on mitotane therapy, whereas

among the 30 patients who had progressive disease, 21 had ongoing mitotane treatment [80]. In comparison to sorafenib, sunitinib was shown to have modest single-agent activity in those who were mitotane naïve, especially because all tyrosine kinase inhibitors are metabolized via CYP3A4, which is induced by mitotane to increase drug clearance [81].

Bevacizumab (an anti-VEGF monoclonal antibody) given at a dose of 5 mg/kg intravenously every 21 days, combined with oral capecitabine (a prodrug of fluorouracil) at a dose of 950 mg/m² twice daily was administered to ten patients with refractory ACC. Fluorouracil has been reported to have adrenolytic properties [82], and capecitabine was chosen because it is an oral agent. All patients had previously received EDP-M or streptozotocin plus mitotane; some patients may have also had prior tyrosine kinase exposure. Additionally, five patients continued to receive concomitant mitotane at the discretion of their local physician. No objective response or stable disease was reported [83]. Some explanations for this could be the doses of bevacizumab and capecitabine used were low due to heavy cytotoxic pretreatment doses.

Unlike sunitinib and sorafenib, which are multikinase inhibitors that target VEGF receptors (VEGFRs) as well as other kinases, axitinib is a selective inhibitor of VEGFR1, 2, and 3. A phase II trial of 13 patients with ACC were given salvage axitinib after previously being treated with at least one chemotherapy and with or without mitotane. Axitinib was started at 5 mg twice daily, with permissible dose titrations (the dose was escalated in 7 patients). No tumor showed a response as defined by the Response Evaluation Criteria in Solid Tumors (RECIST), despite a reduced growth rate in 4 of 13 patients. Median progression-free survival was 5.48 months, and median overall survival was 13.7 months [84].

Although preclinical data suggested that targeting VEGFR was a reasonable option, the VEGFR tyrosine kinase inhibitors applied clinically have not elicited objective RECIST responses; stable disease was the most optimal outcome, and that has only been realized with sunitinib [80].

Other Vascular Targeting Therapies

Lenalidomide is an immunomodulatory, anti-inflammatory, and antiangiogenic drug currently approved for multiple myeloma and some myelodysplastic disorders. Antitumor activity against solid tumors has also been seen, both as a single agent and in combination with other cytotoxic or targeted agents [85]. For instance, in a phase I basket trial, the combination of lenalidomide and the mTOR inhibitor temsirolimus was given to three patients with ACC. Stable disease occurred in two patients and was the best outcome attained [86]. There have been single reports documenting partial responses to other antiangiogenic compounds, such as thalidomide [87], but to date, there have not been more extensive studies in a larger population investigating the efficacy of antiangiogenic agents in the treatment of ACC.

Anti-EGFR Therapy

Epidermal growth factor receptors (EGFRs) have been found to be expressed in over 80 % of ACCs [88, 89], providing a rationale to study agents that target those receptors. Three agents are currently available to block EGFR: cetuximab, erlotinib, and gefitinib [90]. In ACC tumor samples highly expressing EGFR, erlotinib reduced cell viability and induced apoptosis [91], but this pattern was not seen in normal tissue; this suggests that increased sensitivity to EGFR inhibitors is seen when EGFR is highly expressed, such as in ACC.

Compassionate use of erlotinib in combination with gemcitabine (the latter of which was chosen due to its suggested efficacy as a second- or third-line agent at the 2005 ACC consensus conference [59]) was studied in ten patients whose ACC progressed after failing multiple lines of therapy. All patients previously received surgery, mitotane, and at least two cytotoxic chemotherapy regimens. Only 1 patient saw a minimal response, with a progression-free survival time of 8 months; the others all had disease progression. In contrast to the preclinical data, the patient with the least EGFR expression exhibited the greatest response, while the reverse was true

for those who showed the highest EGFR expression [92]. The study therefore demonstrated limited utility for the combination of gemcitabine and erlotinib.

mTOR Inhibitors

Within the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway, the protein kinase mTOR functions as a gatekeeper of cell growth, metabolism, and proliferation. It receives signals from sensors of stress, nutrients, and growth factor receptors [93, 94]. However, the role and function of mTOR in ACC have not been fully clarified [95]. In addition, the expression of mTOR markers varies in ACC. Doghman et al showed that pharmacologic inhibition of mTOR signaling by everolimus greatly reduces childhood adrenocortical tumor cell growth in vitro and in vivo [96]. In another study looking specifically at ACC cell lines, the mTOR inhibitor sirolimus significantly inhibited cell growth in vitro and reduced cortisol overproduction in hormonally active cells [97].

Insulin-Like Growth Factor Receptor Inhibitors

Overexpression of IGF2 is the most common molecular event in ACC and is present in about 90 % of all ACC tumors [98, 99]. IGF2 signals through the IGF1 receptor (IGF-1R), which initiates a downstream signaling cascade driving proliferation, migration, and metastasis of ACC and other cancers [100]. In preclinical studies, antagonizing the IGF-1R pathway with combination mitotane and an IGF inhibitor resulted in greater antiproliferative effects in vitro and in xenograft inhibition in vivo than either agent alone [101]. In a phase I study using figitumumab (a monoclonal antibody against IGF-1R) for refractory ACC, few adverse events were noted but the best outcome was only disease stability [102]. In another phase I trial, the anti-IGF-1R antibody cixutumumab in combination with mitotane showed limited biologic activity [103]. Linsitinib, a

small-molecule inhibitor of IGF-1R and insulin receptor, has shown preliminary evidence of anti-tumor activity in several solid tumors and is well tolerated [104, 105]. In a phase I study specifically of ACC, a partial response was seen [106]. However, in a phase III trial, linsitinib did not improve disease-free or overall survival, though partial responses and disease stabilization were seen in a few patients [107]. Combining an mTOR inhibitor (temsirolimus 25–37.5 mg intravenously weekly) with an IGF-1R inhibitor (cixutumumab 3–6 mg/kg weekly) in 26 heavily pretreated ACC patients (median number of prior therapies 4) resulted in durable stable disease (>6 months) in 11 patients (42%) but no complete or partial objective responses [108]. Thus, despite upregulation of IGF2 in ACC, IGF-1R inhibitors have not been shown to be effective, and further efforts are probably needed to determine who may benefit from this therapy.

cMET Inhibitors

Hepatocyte growth factor (HGF) is known to stimulate tumor angiogenesis by increasing the production of angiogenic cytokines, and it directly activates the cMET receptor, enhancing endothelial cell proliferation and motility [109]. HGF is known to be involved in tumor aggressiveness and resistance to therapy [110]. In pre-clinical studies, *MET* mRNA is upregulated in ACC (especially after chemotherapy or radiotherapy) compared to adrenal adenoma and normal adrenal tissue [55]. In addition, HGF and cMET proteins were increased, leading to increased activation of cMET signaling in ACC and, therefore, proliferation, promotion of angiogenesis, and diminished apoptosis. The reverse effect was also true in *cMET*-knockdown models of ACC. The use of cabozantinib (a powerful multikinase, including cMET, inhibitor) was shown to decrease ACC tumor growth in vitro [55]. Other genitourinary malignancies frequently involve cMET dysregulation, and in a study of cMET inhibitors in patients with such cancers, 21 and 34% had partial response and

stable disease, respectively [111]. In a single case of a patient who developed rapidly progressive disease after multiple therapies failed, cabozantinib was used as a salvage agent. Partial response was seen within a month of treatment and was sustained during subsequent follow-ups at 3 months and 7 months [112]. A clinical trial is needed to explore the role of cabozantinib in the treatment of metastatic ACC.

Immunotherapy

Interleukin-13

Genomic profiling of ACC tissue compared to benign adrenocortical tumors has identified over-expression of several dysregulated genes; among them, interleukin-13 (IL-13) receptor alpha2 (*IL13Ra2*) stands out as a potential marker of malignancy [113]. It has been shown that the cytokine IL-13 promotes invasion through *IL13Ra2* signaling in cell models [114]. Hence, this cell-surface receptor poses an attractive target in treatment strategies. IL-13-PE is a cytotoxin made up of IL-13 and *Pseudomonas* endotoxin (PE) and is cytotoxic to cancer cells expressing *IL13Ra2* in other malignancies [115]. ACC cells have also been shown to be sensitive to IL-13-PE, and the molecule prolonged survival in a mouse xenograft model [114]. In evaluations of this agent's efficacy in other malignancies (e.g., glioma), IL-13-PE was injected directly into the malignant site [116]. In a recent phase I study, however, IL-13-PE was given intravenously to six patients with metastatic ACC in whom standard therapy had failed. All patients had greater than 30% expression of *IL13Ra2*. At the end of the study, only five patients had measurable responses. All patients ultimately had progression of their disease, though one patient had stable disease through six cycles of therapy [117]. There were detectable neutralizing antibodies, which may have diminished the tumor response, and future studies would need to reduce this antibody effect in order to assess the true efficacy of IL-13-PE, especially since there was response in some patients.

Anti-PD-1 Inhibitors

Understanding how the immune system can modulate tumor progression is currently under extensive evaluation. One of the most critical checkpoint pathways responsible for mediating tumor-induced immune suppression is the programmed death-1 (PD-1) pathway. It is normally involved in promoting tolerance and preventing tissue damage in settings of chronic inflammation [118]. Many human solid tumors express PD ligand 1 (PD-L1), and this is often associated with a worse prognosis. Blocking the PD-1/PD-L1 axis using monoclonal antibodies has resulted in promising results in various solid tumors, including melanoma, nonsmall cell lung cancer, and advanced renal cell carcinoma [119–121]; hence, its efficacy in ACC appeared to be appropriate to pursue. Among 28 patients who were surgically treated for ACC, 3 (10.7%) patients had positive PD-L1 staining on tumor membranes, but there was no association between PD-L1 expression and disease stage or overall survival time [122]. The role of immune checkpoints and their therapeutic implications remains

to be determined; currently, 3 phase II studies are assessing the efficacy of nivolumab (NCT 02720484) or pembrolizumab (NCT 02673333, 02721732) for locally advanced or metastatic ACC.

ATR-101

Inhibitors of the acyl-CoA: cholesterol acyltransferase (ACAT) such as ATR-101, which were originally developed for the treatment of atherosclerosis, were also found to have adrenolytic properties [123], in particular in guinea pigs, dogs, and monkeys [124–126], and to reduce adenosine triphosphate (ATP) levels in the adrenal cortex [127]. The observed toxicity of ATR-101 was limited to the adrenal gland, ovaries, and sebaceous glands [126]; hence, it was postulated that ATR-101 might be active against ACC. In cell cultures, xenograft mouse models, and in vivo treatment of dogs, ATR-101 induced cell apoptosis, depleted ATP, and showed evidence of adrenolysis [128, 129]. Currently, ATR-101 is in

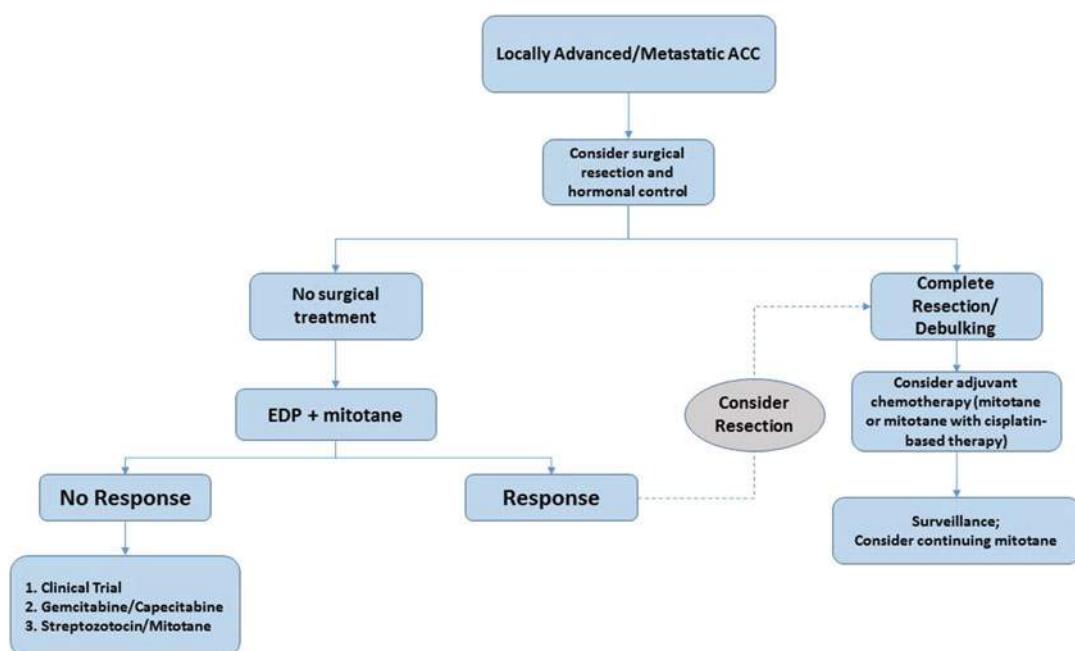


Fig. 14.4 Simplified algorithm summarizing the management of locally advanced and metastatic adrenocortical carcinoma (ACC)

phase I study (NCT01898715) assessing its safety and tolerability in patients with advanced ACC whose cancer progressed on standard therapy. Additionally, due to preclinical suggestions that ATR-101 can affect adrenocortical functions [123, 130], information will also be collected on changes on hormone production, including cortisol, aldosterone, estrogen, and testosterone.

Conclusion

Treating advanced ACC remains a challenge, as summarized in Fig. 14.4. Complete surgical resection, if feasible, carries the most favorable results. Therefore, a patient with locally advanced or metastatic disease should be screened for surgery as primary therapy or after neoadjuvant chemotherapy administered under the supervision of an experienced multidisciplinary team. Depending on the extent of the surgery, adjuvant chemotherapy (with mitotane monotherapy or mitotane combined with a cisplatin-based therapy) should be considered. Where surgery is not feasible, then EDP-M is regarded as the first-line strategy for advanced disease [51]. However, if there is no response to EDP-M, then we recommend considering an alternative systemic therapy, such as gemcitabine–capecitabine or streptozotocin–mitotane, or enrollment in a clinical trial. Though some of the newer targeted therapies have been effective in other malignancies and in isolated cases of ACC, when studied in larger series, many have failed, leaving treatment options that are few in number and confer suboptimal outcomes. With the advent of targeting multiple intracellular pathways and immunotherapy on the horizon, there is hope for a tangible solution to this devastating disease.

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Management of Locally Advanced and Metastatic Pheochromocytoma and Paraganglioma

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Introduction

Pheochromocytoma and paraganglioma are infrequent neuroendocrine tumors usually suspected in patients with endocrine hypertension. Many physicians in the usual clinical practice will not have the opportunity to diagnose or treat a patient with a pheochromocytoma or a paraganglioma and very few clinicians will be in contact with a patient who develops metastases, the hallmark of malignant pheochromocytoma and paraganglioma (MPP). This chapter will discuss the state of the art of MPP to educate physicians in the complex aspects of diagnosis and treatment of these patients and to increase awareness of the close follow-up that is needed in patients with potentially malignant disease. We are currently in

an exciting period as for the first time there are prospective clinical trials being conducted in patients with MPP. The rationale and a few preliminary results from these clinical trials will be discussed in this chapter.

Malignant Pheochromocytoma and Paraganglioma

Pheochromocytoma, a tumor of the chromaffin cells of the adrenal gland, and paraganglioma, a tumor of the chromaffin cells of the paraganglia [1], are rare tumors that occasionally metastasize to other organs such as the bones, liver, and lungs. A histologic, genetic, biochemical, or molecular marker that could be used to differentiate benign pheochromocytoma and paraganglioma from

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malignant pheochromocytoma and paraganglioma has yet to be discovered; therefore, the definition of MPP relies exclusively on the presence of metastatic disease.

Historically, 10% of pheochromocytomas were believed to be malignant; however, recent studies have suggested that the malignancy rates could be as high as 26%. These rates depend on different study variables, such as a referral bias (as MPP patients go to very few institutions with expertise in identifying the disease), follow-up duration, nuclear medicine study availability, and the genetic background of the tumor. For example, small retrospective series have reported malignancy rates as low as 5% [2] in small referral centers and as high as 25.5% in large referral centers [3]. One detailed analysis of the referral pattern in the largest published study of clinical predictors of malignancy indicated that the malignancy rate was about 17% [3].

Pheochromocytoma and paraganglioma metastases most commonly occur in the lymph nodes, followed by the bones [4], liver, and lungs. These tumors may also locally invade the kidneys, pancreas, spleen, inferior vena cava, aorta, and liver given the proximity to the adrenal gland to these structures, and such invasion is considered an indication of aggressiveness. Patients rarely present with metastases in the breasts [3, 5, 6] or skin [7–9]. Patients with MPP have a lower overall survival rate than patients with benign tumors [3]. The reported 5-year survival rates of MPP patients range from 60% [10] to 75.4% [11].

MPP can be classified as synchronous or metachronous disease according to the timing of metastasis presentation [3]. Synchronous metastasis is defined by the presence of metastases at or within 6 months after diagnosis. Metachronous disease is defined by the development of metastases more than 6 months after initial diagnosis. As expected, patients with metachronous disease have a longer overall survival duration than patients with synchronous disease. MPP can also be classified as having a low or high tumor burden [12]. The survival of MPP patients is quite heterogeneous, and patients have variable prognosis; some patients have fast-growing tumors and very short survival durations, whereas others

have tumors that exhibit no or minimal growth over time and have longer survival durations. Survival times vary according to the type and burden of metastases. Patients with bone metastases, high tumor burden, and synchronous disease have shorter survival times [3, 11].

Genetics

For patients with pheochromocytoma and/or paraganglioma, genetic testing should always be considered; this represents an important and well-recognized part of these patients' overall evaluation. Pheochromocytoma and paraganglioma have a significant hereditary burden, and this can be even higher in individuals with malignant disease. Given the medical, familial, and potential psychological implications of identifying an inherited pheochromocytoma/paraganglioma predisposition syndrome, genetic counseling can benefit these patients as they decide on whether or not to pursue genetic testing.

It is estimated that approximately 20–40% of all pheochromocytoma/paraganglioma are associated with an underlying mutation in a pheochromocytoma/paraganglioma susceptibility gene, including apparently sporadic cases [13–17]. Identifying patients with hereditary disease can impact management and surveillance recommendations, and in some cases it can provide prognostic information. Together, this has led to the recommendation that genetic testing should be considered for all individuals diagnosed with pheochromocytoma/paraganglioma, regardless of additional personal and/or family history suggestive of an underlying syndrome [15, 18–21]. This is consistent with the American Society of Clinical Oncology's recommendation that patients with a greater than 10% chance of having a hereditary disease undergo genetic testing [22]. The likelihood of identifying an inherited predisposition syndrome can be even higher in individuals who have malignant pheochromocytoma/paraganglioma, with up to 50% of cases being due to mutations in *SDHB* [17, 23–25].

To date, 14 pheochromocytoma/paraganglioma susceptibility genes have been identified and

the potential role of additional genes in the development of pheochromocytoma/paraganglioma is being investigated (e.g., *KIF1Bβ* and *EGLNI*) [26, 27]. The susceptibility genes include *VHL*, *NF1*, and *RET*, which are each associated with a well-described syndrome that, along with other benign and malignant tumors, predisposes to pheochromocytoma/paraganglioma, and the following pheochromocytoma/paraganglioma syndrome genes: *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *TMEM127*, *FH*, and *MAX*. Several algorithms have been developed to help clinicians decide on which genes to test for in patients based on characteristics such as tumor location, biochemical phenotype, syndromic presentation, and/or evidence of metastases [15, 18, 20, 27]. To provide an accurate genetic risk assessment in this setting, it is important to consider all aspects of a patient's histories, but the focus herein will be on how having a malignant tumor which can impact the approach to genetic testing and counseling.

Mutations in *SDHB* are significantly associated with MPP and it is recommended that patients with malignant disease first be offered at least *SDHB* testing [18, 28–30]. The penetrance of all pheochromocytoma/paraganglioma with *SDHB* mutations has been estimated to be as high as 92% [31] and the risk of malignancy approximately 34% [32]. *SDHB* mutations have been identified in apparently sporadic cases of benign pheochromocytoma/paraganglioma as well, suggesting that evidence of metastases and/or a positive family history should not be the only scenario in which *SDHB* testing is initially considered [17, 33]. Identifying benign cases of pheochromocytoma/paraganglioma caused by *SDHB* mutations can provide useful prognostic information for the clinician with regards to risk for later metastatic disease in both index patients and their family members. It is important to recognize the increased incidence of extra-paraganglia tumors, although not common manifestations, associated with *SDHB* mutations including renal cell carcinoma (RCC), gastrointestinal stromal tumors (GISTs), and possibly nonmedullary thyroid cancer [32, 34, 35]. Malignant PCC/PGL have also been reported in association with *SDHD* mutations, but the overall risk for malignancy is less

than 5% [31, 34]. *SDHC*-related tumors are almost always benign [36, 37] and there were no reports of malignant tumors in a large kindred with an *SDHAF2* mutation [38]. The phenotype and potential risk for malignancy of *SDHA*-related PCC/PGL is not well defined to date.

MPP have been reported in patients with von Hippel–Lindau disease (VHL), Neurofibromatosis type 1 (NF1), and multiple endocrine neoplasia type 2 (MEN2). The frequency of MPP in each syndrome, respectively, is approximately 5%, 12%, and less than <1% [39–42]. Each of these syndromes is characterized by an increased risk for pheochromocytoma, though paraganglioma has also been reported, and additional benign and malignant tumors [43–45]. Paragangliomas do not occur in patients with MEN2 [42]. The presence of an apparently sporadic, MPP would rarely prompt *NF1* or *RET* testing alone; however, it has been recommended that *VHL* testing be considered in individuals with apparently sporadic, MPP who have no identifiable *SDHB* mutation [28].

More recently, *MAX* and *TMEM127* have been identified as pheochromocytoma/paraganglioma susceptibility genes and *MAX*-related tumors have been associated with an increased risk for malignancy; initial studies investigating the phenotype of *MAX*-related pheochromocytoma/paraganglioma found malignant tumors in 10–37% of mutation carriers [46–48]. These studies were in small cohorts and further research is needed to elucidate the overall penetrance of and risk for malignancy associated with *MAX* mutations; nevertheless, the risk for malignancy is greater in *MAX* compared to, aside from *SDHB*, all other PCC/PGL susceptibility genes. Clinical *MAX* mutation testing is still relatively new and the frequency of disease-causing gene mutations identified among patients with MPP may increase as more of these patients undergo both *SDHB* and *MAX* testing. There has been one report of a MPP in an individual with a *TMEM127* mutation [49].

Genetic testing for individuals with malignant PCC/PGL should include at a minimum *SDHB*, and evidence is growing to support considering *MAX* testing as a first tier test as well. If the patient's history, biochemical phenotype, or location of the tumor(s) is characteristic of a syndromic presentation

or another pheochromocytoma/paraganglioma susceptibility gene (e.g., bilateral carotid body tumors often prompt *SDHD* testing), then that testing should also be initially considered. The development of Next-generation sequencing (NGS) panels has allowed for the simultaneous analysis of multiple pheochromocytoma/paraganglioma susceptibility genes, which can be beneficial in some cases but perhaps not necessary in all. Multiple commercial laboratories offer genetic testing for ten pheochromocytoma/paraganglioma susceptibility genes, with options to test all genes simultaneously and/or the ability to customize an NGS panel based on risk assessment and patient preference. Regardless, the patient should be involved in the decision-making to pursue genetic testing and healthcare teams that manage individuals with pheochromocytoma/paraganglioma should be prepared to order germline genetic testing.

This preparation should include (1) an awareness and understanding of currently available genetic testing options for patients with pheochromocytoma/paraganglioma and their respective sensitivities, technical limitations, etc. (detailed information about the laboratories that perform genetic testing can be found in the National Center for Biotechnology Information's Genetic Testing Registry®); (2) the ability to perform an individualized risk assessment to determine which genes to consider testing for in each patient; and (3) the provision of pretest and post-test counseling for patients and their family members. Both the National Comprehensive Cancer Network's Clinical Practice Guideline for Neuroendocrine Tumors (Version 1.2016) and the American Endocrine Society's Clinical Practice Guidelines for pheochromocytoma/paraganglioma [18] mention genetic counseling as part of the consideration for genetic testing in patients with pheochromocytoma/paraganglioma, citing the benefit of counseling for informed decision-making and to ensure an understanding of the implications of genetic test results can have on patients and/or their family members.

Patients with MPP undergoing genetic testing should be aware of the potential to identify mutations in genes associated with other tumor and/or cancer risks, as this could impact future medical

management and surveillance recommendations for themselves and their family members, including young children [50]. The potential to receive and limitations of inconclusive genetic test results (i.e., variants of unknown significance or variants of unknown clinical significance) should be addressed with any patient prior to genetic testing, especially as more NGS panels allow for simultaneous analysis of multiple pheochromocytoma/paraganglioma susceptibility genes are made available, which in some cases may be the preferred method of testing [51]. It is also important that patients have the opportunity to consider genetic testing issues related to insurance, including coverage, out-of-pocket costs, and the impact results could have on insurability [52]. Last but not least, the potential psychosocial impact of genetic testing for hereditary diseases should be explored with each patient given the potential for certain results to cause emotional distress in some patients and family members [53].

Each of the known hereditary pheochromocytoma/paraganglioma syndromes is inherited in an autosomal dominant pattern and can demonstrate both variable expressivity and penetrance. At-risk family members of individuals with a mutation in a pheochromocytoma/paraganglioma susceptibility gene should be offered predictive genetic testing. Uniquely, mutations in *SDHD*, *SDHAF2*, and *MAX* are imprinted, the risk for pheochromocytoma/paraganglioma is apparent in offspring when the mutation is inherited paternally but one needs to be cautious when counseling patients identified to have such mutations in these genes as this phenomenon has not been definitively proven in all cases [32, 34, 38, 47]. Children whose mothers have a mutation in one of these imprinted genes should still be counseled about the chances of passing them on, especially in the case of unaffected male carriers whose children would have a high risk of developing pheochromocytoma/paraganglioma if the mutation was inherited. Thus, genetic testing should still be offered to individuals who potentially inherited these mutations from their mother. This unique inheritance pattern is especially important to consider during risk assessment and counseling for patients with MPP, as they may be more likely to undergo *MAX* testing

and subsequently test positive for a *MAX* mutation. Mutations in *SDHB*, *SDHC*, and *SDHD* have been identified in patients with Carney–Stratakis syndrome, characterized by an increased risk for GISTs [54]; biallelic *SDHA* and *SDHB* mutations can cause autosomal recessive mitochondrial diseases, including Leigh syndrome and leukodystrophy [55, 56].

The potential for hereditary disease in patients with MPP is significant and all of these patients should be given the opportunity to pursue genetic testing. Knowledge about pheochromocytoma/paraganglioma genetics is rapidly evolving and it is important to stay aware of the salient changes in this field that can impact genetic risk assessment, testing, and counseling of patients.

Predictors of Malignancy

Several studies have attempted to identify clinical and histopathological findings that indicate the malignant potential of pheochromocytoma and paraganglioma. Because the information derived from histological analyses is not standardized, difficult to reproduce, and variable, clinical predictors of malignancy are used to determine the need for long-term follow-up, the imaging studies to evaluate extent of disease, and the initial surgical approach.

Clinical Predictors of Malignancy

The three well-recognized clinical predictors of malignancy in pheochromocytoma and paraganglioma patients are primary tumor size, primary tumor location, and presence of inactivating germline mutations in the *SDHB* gene.

Primary Tumor Size

Primary tumor size is a predictor of metastasis [3, 57] and is associated with survival. A primary tumor size of more than 5 cm is associated with a higher risk of metastasis and a lower survival duration, and patients with such tumors must have lifelong follow-up [11].

Primary Tumor Location

Primary tumor location is a main predictor of metastasis. Patients who have sympathetic paragangliomas (tumors located in the thoracic, abdominal, or pelvic cavities) have higher malignancy rates than do patients who have pheochromocytomas or parasympathetic paragangliomas. In fact, patients with sympathetic paragangliomas may have a malignancy rate as high as 70 %, whereas patients with pheochromocytomas or head and neck paragangliomas may have malignancy rates of only 15–17 % and less than 5 %, respectively. One retrospective study from MD Anderson Cancer Center showed that patients with sympathetic paragangliomas have a high risk of metastatic disease (odds ratio: 4.5; 95 % confidence interval [CI]: 2.8–7.3), which suggests that such patients need lifelong follow-up. Patients with mediastinal, abdominal, or pelvic paraganglioma have a 5-year risk of new event, recurrence, or metastasis of 18 %. Independent of *SDHB* mutations, which exhibit a phenotype frequently characterized by the presence of sympathetic paragangliomas, primary tumor location is associated with a higher risk of death than *SDHB* mutations [3].

Germline *SDHB* Mutations

An initial characterization of the clinical phenotype associated with *SDHB* mutations revealed that up to 50 % of pheochromocytoma and paraganglioma patients with such mutations may have metastatic disease. Furthermore, Amar et al. [58] found that patients with MPP associated with *SDHB* mutations may have a lower survival rate than patients with apparently sporadic MPP. This finding needs to be confirmed in larger, ideally prospective studies, as our clinical experience suggests that patients with sporadic disease can also have very aggressive tumors [3]. Compared with patients who have a single, apparently sporadic pheochromocytoma or paraganglioma, those with *SDHB* mutation-associated tumors are more frequently screened for metastatic disease suggesting a selection bias.

Germline *FH* Mutations

One gene recently discovered to be associated with MPP is the fumarate hydratase (*FH*) gene, *FH*, which encodes for the *FH* enzyme [59]. Castro-Vega et al. found that 5 pheochromocytoma and paraganglioma patients with *FH* mutations had malignant disease [60]. However, in another study, Clark et al. found that patients with *FH* mutations did not have malignant disease, even after a follow-up duration of at least 5 years [61]. Cases of *FH* mutation-associated pheochromocytoma and paraganglioma are rare, and further studies are needed to confirm that *FH* mutations are indeed strongly associated with an increased risk of malignancy.

Pathological and Molecular Markers of Malignancy

There is an increased need to determine on initial histological evaluation whether a pheochromocytoma or paraganglioma has a malignant potential. Unfortunately, there are no histological characteristics that indicate the malignant potential. From a therapeutic perspective, a clear histological characterization could clarify whether adjuvant therapy will be needed after resection of the primary tumor, for instance.

Several pathological and molecular markers of malignancy have been proposed. Some consider a Ki-67 index of more than 3 % to be a specific marker of malignant disease [62, 63], and several somewhat limited retrospective studies support this assertion [64–68]; in one of these studies, increased Ki-67 expression was considered to be a very strong predictor of malignant disease [63]. However, another study found no correlation between Ki-67 expression and malignancy [69]. In addition, some pheochromocytoma and paraganglioma patients can have very aggressive tumors with no or low Ki-67 expression, whereas other patients can have nonmetastatic very slow-growing tumors with Ki-67 indexes of more than 3 %. Thus, additional studies to determine the value of Ki-67 as a predictor of malignancy in pheochromocytoma and paraganglioma patients are needed.

Two pathological scoring systems have been proposed to identify malignant disease: The Pheochromocytoma of the Adrenal Gland Scaled Score [70] and the Grading System for Adrenal Phaeochromocytoma and Paraganglioma [71]. Both systems take into account the Ki-67 index and other markers (e.g., those that might suggest poor histological differentiation); however, neither system has been validated for the diagnosis of malignancy. Furthermore, these systems' scoring criteria can be quite subjective and very difficult to reproduce in clinical practice [72]. In addition, even patients with well-differentiated tumors can have metastatic disease. Therefore, using the Pheochromocytoma of the Adrenal Gland Scaled Score or Grading System for Adrenal Phaeochromocytoma and Paraganglioma to determine malignant potential is not recommended, as these systems are unreliable and could have a negative impact on clinical practice. Long-term prospective studies in different populations are required to properly establish the role of histological markers in the management of pheochromocytoma or paraganglioma.

Other reported pathological and molecular markers of malignancy that require further evaluation include tumor necrosis [73], intracytoplasmic hyaline globules [73], high mitotic rate, SDHB immunochemistry, extracellular matrix metalloproteinase inducer (EMPRINN), human telomerase reverse transcriptase [74], zinc-finger transcription factor (SNAIL), EM66, mm-23, cyclooxygenase-2 (COX-2), Galectin-3, insulin-like growth factor 2, microRNA (miR)-483-5p, miR-183, and miR-101 [75].

Hormonal Markers of Malignancy

Patients with clinical suspicion of pheochromocytoma and paraganglioma such as episodic hypertension, tachycardia, or incidental adrenal mass must have biochemical evaluation with 24-h fractionated metanephrenes or plasma total metanephrenes. Although metanephrene levels more than three times the upper limit of normal are almost always considered diagnostic of pheochromocytoma or sympathetic paraganglioma

[18], it is possible to see patients with these tumors in whom metanephrine levels are mildly elevated. Patients with resected pheochromocytoma or paraganglioma should have measurement of plasma or urine levels of metanephries in the clinical follow-up to detect recurrent disease or metastatic disease.

Patients with already diagnosed metastatic disease may have lower tumor burden, and thus lower metanephrine levels, therefore, any elevation in metanephrine levels should be pursued properly with imaging studies. In addition, because pheochromocytoma and paraganglioma metastases can be nonfunctional or small, and thus have no or minimal effect on metanephrine levels, patients with malignant or suspected MPP should also undergo serial imaging studies as part of their follow-up or initial staging workup.

Evaluating the hormonal production profile is important and may suggest localization of the disease. Tumor production of both metanephrine and normetanephrine is highly indicative of an adrenal tumor site. Sympathetic paragangliomas lack the enzyme phenylethanolamine-*N*-methyltransferase, which converts noradrenaline to adrenaline; this is why paragangliomas have a hormonal profile of increased normetanephrine. As metastatic disease is more common with sympathetic paragangliomas, patients with these tumors more frequently have increased levels of normetanephrine. In addition, the concentration of total metanephries is correlated with primary tumor size and, in malignant disease, with tumor burden [76].

If pheochromocytoma or paraganglioma is metastatic, it does not necessarily mean that it is dedifferentiated, and most patients with metastatic disease have a hormonal profile reminiscent of that of patients with nonmetastatic disease. However, increased levels of 3,4-dihydroxyphenylalanine, dopamine, and plasma 3-methoxythiramine [77] have been put forth as markers of metastatic disease [78], which suggests that metastatic tumors lack the enzymes capable of converting 3,4-dihydroxyphenylalanine to catecholamines. However, these data must be carefully interpreted, as they are from a retro-

spective study that did not follow the “Reporting recommendations for tumor marker prognostic studies” (REMARK) criteria [79] and was associated with a referral bias. Therefore, prospective studies performed in other centers are needed to clarify whether 3,4-dihydroxyphenylalanine, dopamine, and plasma 3-methoxythiramine are useful markers of malignant potential in pheochromocytoma and paraganglioma patients.

Role of Imaging Studies

Imaging studies are usually used to detect metastases in pheochromocytoma and paraganglioma patients and thus diagnose MPP. Patients who have a biochemical diagnosis of pheochromocytoma or an apparently sporadic pheochromocytoma and have a single mass smaller than 5 cm revealed by abdominal computed tomography (CT) or magnetic resonance imaging (MRI) do not need to undergo additional imaging studies. However, patients with predictors of malignancy, such as a mass larger than 5 cm, *SDHB* mutation-associated tumors, an extra-adrenal tumor location, or multifocal disease, must undergo additional imaging studies and/or other tests to detect metastases and determine whether the disease, if metastatic, will respond to targeted therapy such as ^{131}I -metaiodobenzylguanidine (MIBG).

The ideal initial imaging study for pheochromocytoma and paraganglioma patients is not well established and depends on several aspects, such as genetic background and tumor location. Most patients undergo at least one conventional imaging study, such as CT or MRI (Fig. 15.1), which detect metastases with greater than 95% sensitivity. All patients should also undergo at least one functional imaging study, such as ^{131}I -MIBG scintigraphy (Fig. 15.2) or 2-deoxy-2-[fluorine-18] fluoro-D-glucose (^{18}F -FDG) positron emission tomography (PET)/CT (Fig. 15.3). Patients who have *SDHB* mutations should undergo ^{18}F -FDG PET/CT, as this imaging modality detects metastases with 100% sensitivity. However, ^{18}F -FDG PET/CT can yield false positives (e.g., infectious/inflammatory changes) and may not detect metastases in

Fig. 15.1 CT scan showing the initial tumor in a 41-year-old woman with neurofibromatosis and malignant pheochromocytoma. Large left adrenal mass (arrow) that is heterogeneous and invading the kidney. After the resection of such masses, patients should have close follow-up with functional imaging and at least annual measurement of catecholamines

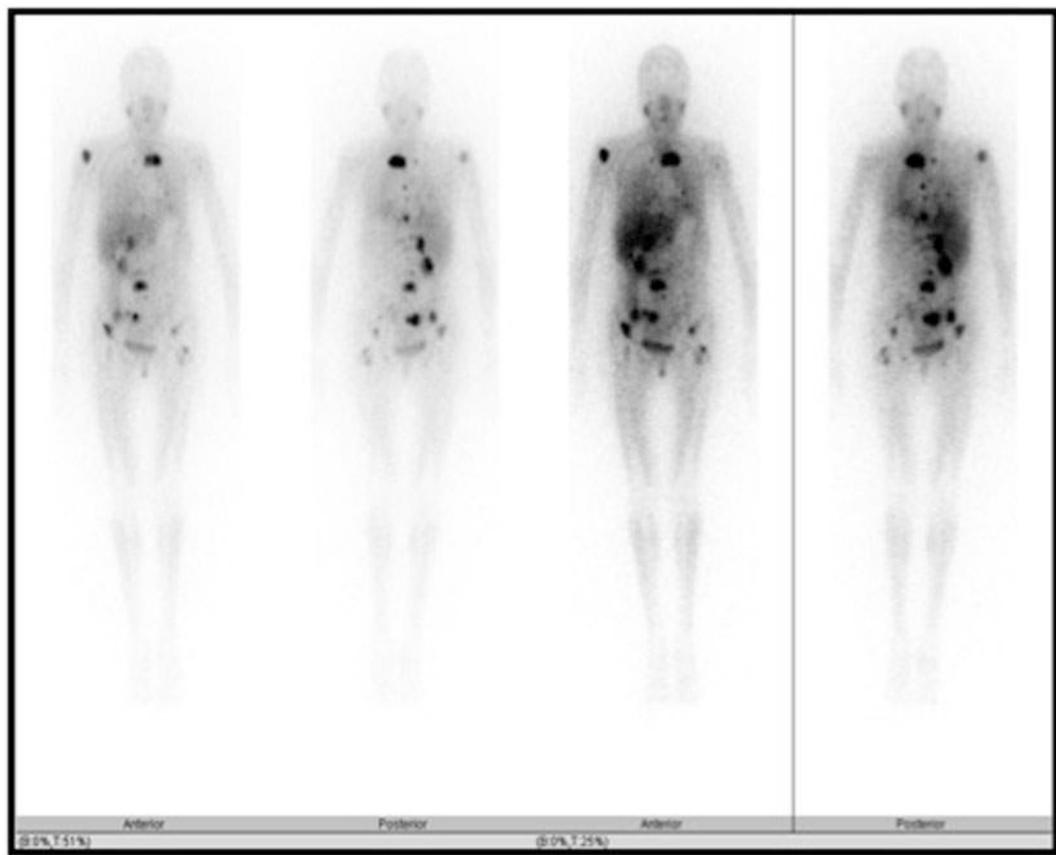
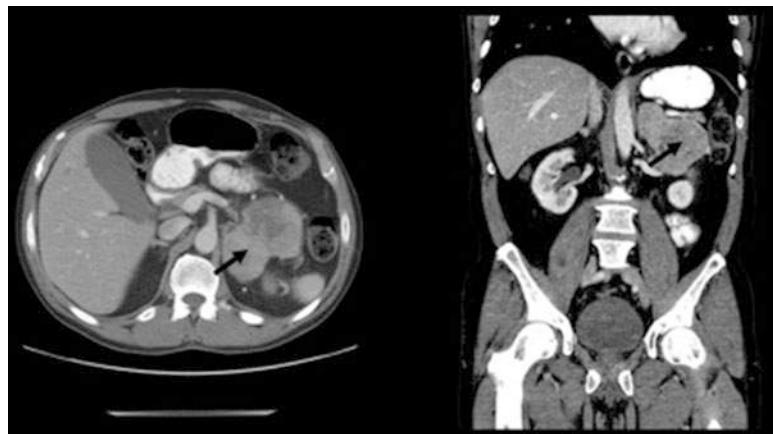
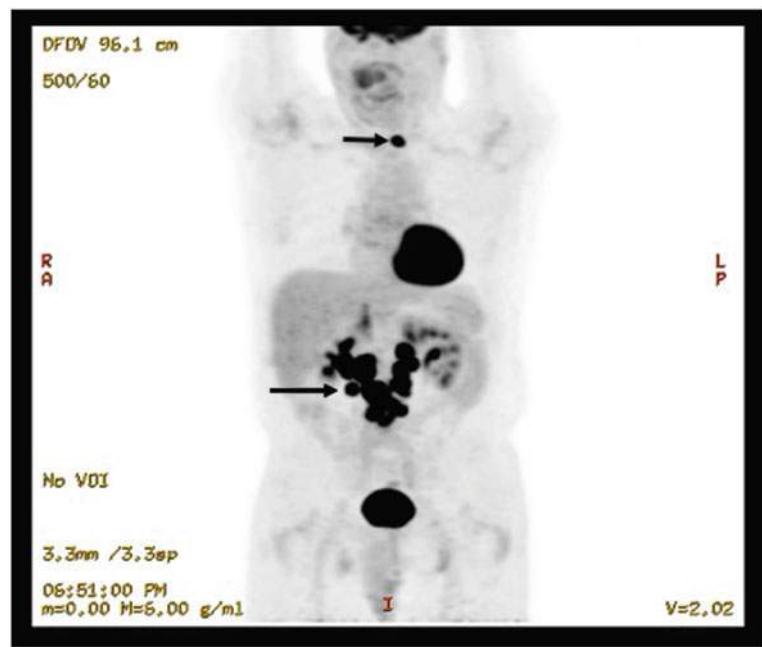


Fig. 15.2 ^{123}I -MIBG scintigraphy in a 41-year-old woman with neurofibromatosis and malignant pheochromocytoma. Abnormal activity in the right upper abdominal quadrant corresponds to local recurrence. Multiple foci of abnormal activity are consistent with metastatic

disease sites; foci in the spine, left posterior fourth rib, pelvis, right proximal humerus, and right kidney region. A peritoneal soft-tissue nodule demonstrating MIBG uptake is consistent with peritoneal metastasis

Fig. 15.3 ^{18}F -FDG PET/CT of a 53-year-old man with a *SDHB* positive malignant pheochromocytoma



patients with apparently sporadic MPP. ^{131}I -MIBG scintigraphy can provide some very useful information with regards to therapy, as ^{131}I -MIBG-avid tumors may respond to therapy with ^{131}I -MIBG. In addition, ^{131}I -MIBG scintigraphy may detect metastases that are not detected by ^{18}F -FDG PET/CT.

Bone metastases are quite common in MPP patients [4] and are associated with pain, hypercalcemia, and fractures. Systemic therapy and bone-directed therapies decrease skeletal-related events. Bone scanning should be performed to rule out this type of metastasis in all patients with malignant disease [10].

MRI is generally recommended as the follow-up imaging modality, as it limits patients' long-term exposure to radiation.

The role of imaging studies with new somatostatin analogs is unclear and has not been systematically evaluated in MPP patients. The most promising of these modalities is ^{68}Ga -DOTANOC PET/CT [80], which detects malignant pheochromocytoma in patients with MEN2 syndrome with 100% sensitivity and better accuracy than ^{131}I -MIBG scintigraphy.

Treatment

Owing to their large tumor burden and excessive secretion of catecholamines, MPPs are associated with high rates of morbidity and mortality. MPP patients have a median 5-year overall survival rate of 60 %. In this context, MPP patients may be offered one of two initial approaches: active surveillance or systemic therapy (i.e., chemotherapy, radiopharmaceutical therapy, molecular targeted therapy, and/or immunotherapy).

An active surveillance approach for MPP patients is possible; in fact, a subgroup of MPP patients has very slow disease progression, prolonged survival, and no or minimal symptoms that can be controlled with alpha- and beta-blockers. Currently available systemic treatments for MPP are palliative; the main goals in using such therapy in MPP patients are to prolong progression-free survival and improving quality of life. For patients with slow-growing MPP, systemic therapies such as chemotherapy may add unnecessary toxicity without having a positive

impact on the patient's clinical outcome [12] and thus may not be indicated. For these patients, clinical and radiographic follow-up every 3 months for 1 year upon initial diagnosis and every 6–12 months thereafter is recommended. At some point, most of these patients will have disease progression at which point intervention with systemic therapies could be considered [12, 81] (Fig. 15.4).

Systemic Therapy

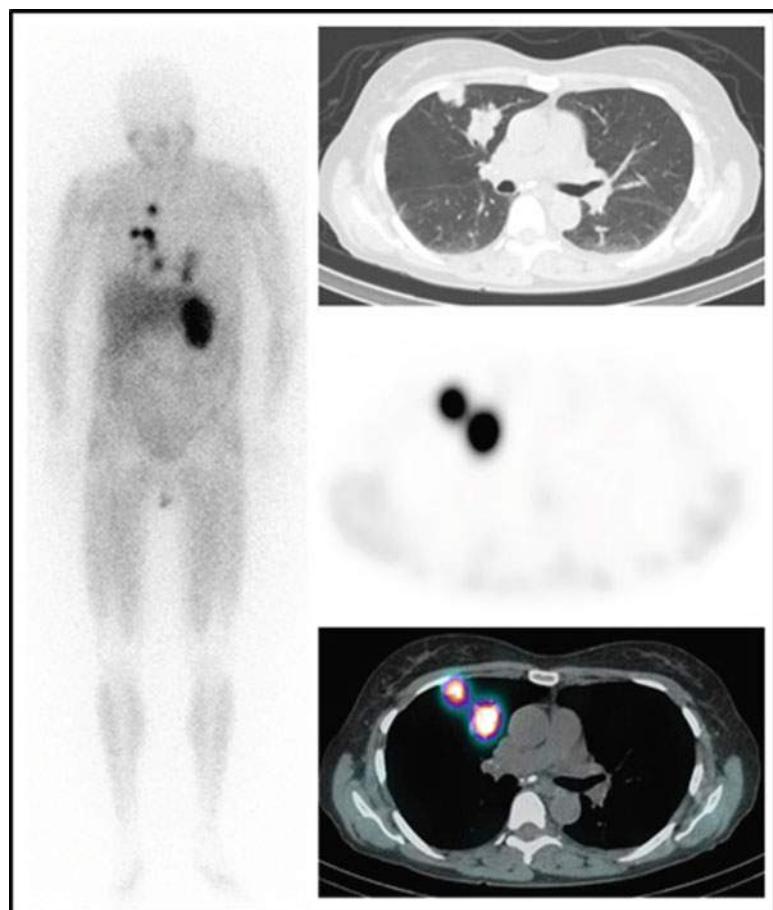
One of four MPP characteristics may justify the use of systemic therapy:

1. Symptoms of catecholamine excess that are not controlled by alpha- and beta-blockers.
2. Symptoms related to tumor burden and growth.

3. Large tumor burden defined as 7 or more bone metastases, replacement of 50 % or more of the liver parenchyma, and/or multiple lung nodules larger than 2 cm.
4. Disease progression as defined by RECIST 1.1 [12] over a period of 3–6 months.

In general, systemic therapies have been associated with partial radiographic responses, disease stabilization, and symptom improvement in approximately 40 % of MPP patients. Of the currently available treatments, only systemic chemotherapy has been associated with a modest overall survival improvement, as described by a single retrospective study (the largest one published to date) [10]. Unfortunately, given the rarity of the disease, no published studies provide guidance on how to sequence treatments to obtain the best possible progression-free survival, overall survival, and quality of life in MPP patients.

Fig. 15.4 ^{123}I -MIBG imaging in a 56-year-old female with malignant pheochromocytoma. *Left:* Anterior whole-body ^{123}I -MIBG planar image demonstrating multiple foci of abnormally increased intrathoracic uptake. *Right:* CT (top), SPECT (middle), and fused SPECT/CT (bottom) images demonstrate foci of uptake corresponding to pulmonary nodules consistent with lung metastases



Chemotherapy

Systemic chemotherapy has been used to treat MPP patients since the early 1960s. Given the rarity of the disease, our understanding of the use of systemic chemotherapy in MPP patients is derived from small, retrospective studies of different chemotherapy regimens (e.g., cyclophosphamide, vincristine, doxorubicin, and dacarbazine (CyVADic), cyclophosphamide, adriamycin and vincristine (CyAV), cyclophosphamide, adriamycin (CyA), Cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone (CHOP) (Table 15.1) [82–85]. These studies used different parameters to assess therapeutic response [10, 12] and included different regimens consisting of at least two chemotherapeutic agents. The most common regimen used to treat MPP patients includes cyclophosphamide, vincristine, and dacarbazine (CVD) with or without doxorubicin. Studies evaluating this regimen and its variants have described response rates ranging from 25 to 55% [12, 82, 85]. Complete responses were reported in a small retrospective study by Huang et al. but not in larger, more recent studies by Ayala-Ramirez et al. and Hadoux et al. [12, 82, 85].

In their retrospective study, Ayala-Ramirez et al. found that MPP patients who received systemic chemotherapy had a 5-year overall survival rate of 51%. The authors classified patients as responders or nonresponders based on (1) the extent of tumor size reduction on conventional radiography and (2) the extent of blood pressure control as determined by reductions in the dose and number of antihypertensive medications. The median overall survival duration of the responders (6.4 years) was longer than that of the nonresponders (3.7 years), but this difference was not significant ($p=0.095$). However, a multivariate analysis adjusting for tumor size at the time of diagnosis showed that the difference in survival was significantly longer ($p=0.05$; HR: 0.22; 95% CI: 0.05–1.0). This study was the first to suggest that chemotherapy offers a survival benefit in MPP patients.

Patients whose disease responds to CVD chemotherapy are not cured of the disease, which is unsurprising given the large tumor burden that

characterizes MPP. Some oncologists have advised the long-term use of CVD chemotherapy to control the disease and prevent tumor resistance; however, the side effects associated with CVD and other regimens are not negligible and may negatively impact some patients' quality of life. Doxorubicin has a limiting dosage, and the chronic use of vincristine can cause peripheral neuropathy and predispose patients to constipation, a complication to which patients with hormonally active MPP are already susceptible to [86]. Furthermore, rare cases of secondary leukemia, myelodysplastic syndromes, and Sweet syndrome in patients treated with long-term CVD chemotherapy have been reported. In patients who initially respond to CVD chemotherapy, a maintenance regimen with dacarbazine or temozolomide and discontinuation of the other medications could be implemented after 6 cycles of chemotherapy as long as there is evidence of a partial radiographic response or disease stabilization in association with clinical improvement. Such a maintenance regimen, which is similar to that used in patients with colon, lung, and breast cancers, could reduce the rate of permanent side effects, could provide an acceptable quality of life, and might be associated with progression-free survival. In addition, given the very few therapeutic options available, this approach would allow for previously discontinued first-line medications to be reintroduced at the time of disease progression thereby reserving the use of second-line therapies for later.

Other chemotherapy regimens consisting of agents including cisplatin, 5-fluorouracil, methotrexate, ifosfamide, and streptozotocin [10] have been used in occasional MPP patients; however, no strong evidence supports these regimens' use in clinical practice.

In regards to adjuvant chemotherapy, there is still no scientific evidence that could indicate its use; this is different from what we currently do for patients with well-differentiated neuroendocrine tumors derived from the gastrointestinal tract as they may benefit from adjuvant treatment. It is not clear if for patients with any of the three clinical predictors of malignancy —extra-adrenal tumor location, a primary tumor larger than 5 cm, and the presence of *SDHB* mutations [10]—the

Table 15.1 Summary of retrospective series of chemotherapy that included at least 15 patients with malignant pheochromocytoma and paraganglioma

Study	No. of evaluable patients/total number of patients	Chemotherapy regimen	Mean no. of cycles	Methodology	Response rate	Mean response duration, months	Overall survival
Huang et al. [82]	18	C (750 mg/m ² , day 1) V (1.4 mg/m ² , day 1) D (600 mg/m ² , days 1 and 2)	18	Retrospective analysis, WHO-like criteria	55% CR or PR	20	3.3 Years
Ayala-Ramirez et al. [83]	52/54	C (600–750 mg/m ²) D (750–1000 mg/m ²) +/- Dox (60–75 mg/m ²) +/- V (1–2 mg/m ²)	6.9	Retrospective analysis, nonstandardized	25% PR	Unknown	51% 5-year survival rate
Tanabe et al. [84]	17/23	C (750 mg/m ² , day 1) V (1.4 mg/m ² , day 1) D (600 mg/m ² , days 1 and 2)	Unknown	Retrospective analysis, nonstandardized	47% molecular response or PR	40	Unknown
Hadoux et al. [85]	15/15	TMZ (150–200 mg/m ² , days 1–5)	7	Retrospective analysis, RECIST 1.1, PERCIST 1.0	33% PR	13	55% 5-year survival rate

From Baudin et al. [12], with permission.

C cyclophosphamide; V vincristine, D dacarbazine, WHO World Health Organization, CR complete response, PR partial response, Dox doxorubicin, TMZ temozolomide, RECIST response evaluation criteria in solid tumors, PERCIST positron emission tomography response criteria in solid tumors

use of 4–6 cycles of CVD chemotherapy after surgery may decrease the chances of local and/or distant disease progression or recurrence (such an approach might also improve progression-free survival and overall survival). However, whether patients with clinical predictors of malignancy would indeed benefit in these ways must be investigated in randomized phase III clinical trials.

Radiopharmaceutical Therapy

Metaiodobenzylguanidine, also known as iobenguane, mIBG or MIBG, is a guanethidine derivative resembling norepinephrine which is taken up by cells in the sympathomedullary system. MIBG is a semiselective agent for malignant pheochromocytoma and paraganglioma which can be used for both diagnostic and therapeutic purposes by labeling with radioisotopes of iodine. When formulated with ^{131}I , MIBG can be used to treat patients with catecholamine-secreting metastases which demonstrate uptake on MIBG imaging. The therapeutic effect is achieved through the beta-minus decay of ^{131}I causing radiation damage to target tissue which has been shown to lead to treatment response by both anatomic imaging and biochemical criteria [87].

Both ^{123}I and ^{131}I -MIBG can be used for imaging purposes [88]. Whole-body planar imaging (two-dimensional) is performed following the intravenous administration of ^{123}I or ^{131}I -MIBG allowing for assessment of MIBG-avidity of tumors and assessment of the extent of metastatic disease. SPECT (single photon emission computed tomography) imaging can also be performed to provide three-dimensional distribution of activity. With some modern equipment, SPECT/CT images can be obtained which combined SPECT with CT (computed tomography) giving added anatomical detail and aiding in the localization of foci of uptake (Fig. 15.4). ^{123}I is typically preferred for imaging given the lack of beta emissions and shorter physical half-life (13.2 h versus eight days) resulting in overall lower radiation exposure to the patient. The gamma emission of ^{123}I is of lower energy than

that of ^{131}I and is more optimal for imaging with conventional gamma cameras. For purposes of dosimetry, however, ^{131}I -MIBG is preferable to ^{123}I -MIBG as images can be obtained at multiple time points over several days which can aid in the estimation of radiation-absorbed dose to specific organs.

^{131}I -MIBG therapy is not currently FDA approved for treatment of MPP but has been evaluated in a number of relatively small clinical trials. Appropriate regimens of ^{131}I -MIBG have not yet been established. There have been diverse approaches to treatment regimens with respect to the amount of administered activity per treatment cycle, total number of treatments, and treatment intervals. These approaches can be categorized into two basic strategies using either a limited number of high-dose treatments or multiple relatively low-dose treatments. Both high-dose regimens and multiple fractionated doses have both been shown to be effective in achieving therapeutic response [89].

The existing evidence suggests that ^{131}I -MIBG may be used as first-line therapy in patients with strongly positive ^{123}I -MIBG scintigraphy findings, who have significant tumor burden with slowly progressive disease and almost normal blood counts and appropriate kidney function.

^{131}I -MIBG therapy may control the tumor burden and/or the catecholamine excess and may improve survival. The limitations of using this therapy in clinical practice include its safety and availability. However, the role of this therapy in MPP patients is still relatively unclear, as no randomized clinical trials have been performed and most of the evidence supporting its use is retrospective.

A meta-analysis has been performed including 17 separate studies published from 1984 to 2012 including 243 patients with MPP with mean duration of follow-up ranging from 24 to 62 months. Patients were treated with a wide range of both cumulative activity and number of treatments. Median cumulative administered activity ranged from 6882 to 39,400 MBq (186–1065 mCi) with a median number of infusions ranging from 1 to 7. The results of the meta-analysis found a complete response in 3%, partial response in 27%, and

stable disease in 52 % of treated patients. Biochemical response was reported as 11 % complete response, 40 % partial response, and 21 % stable disease [90]. Patients with involvement limited to soft tissues have been reported to achieve better objective responses compared to those with bone metastases [91].

Few studies have investigated the survival outcomes of patients who received ^{131}I -MIBG. Gonias et al. [92] and Safford et al. [93] reported 5-year survival rates of 64 and 45 %, respectively. Hormonal and symptomatic responses were correlated with improved survival as well as a high dose of ^{131}I -MIBG. Gedik et al. [94] reported a median progression-free survival time of 24 months (range, 3–93 months), and Shilkret et al. [95] reported a median progression-free survival time of 17.5 months.

At relatively lower doses the treatments are generally well tolerated, the most common adverse effects consisting of nausea, vomiting, and anorexia as well as mild leukopenia and thrombocytopenia [96]. In the aforementioned meta-analysis, hematologic toxicity was the most frequently reported side effect of ^{131}I -MIBG. However, grade 3 or 4 neutropenia and grade 3 or 4 thrombocytopenia were also common. Patients may also develop hematologic malignancies such as myelodysplasia and acute or chronic myeloid leukemia [97]. One uncommon complication of ^{131}I -MIBG is renal failure, which is usually mild and occurs in patients with other factors that have a deleterious effect on kidney function, such as diabetes or hypertension. Other possible side effects are asthenia, nausea, vomiting, hypertensive crisis, sepsis, and pulmonary toxicity [12].

At higher doses, sustained complete response has been reported in a small number of patients, although with an increased risk of potentially serious adverse effects. In one study involving 12 patients who received high-dose therapy, patients received a median cumulative administered activity of 1015 mCi. Complete response was achieved in three patients (two of whom had soft tissue and skeletal metastases). Seven patients achieved a partial response, and two

patients with no response died with progressive disease. Grade 3 thrombocytopenia occurred following 79 % of treatments. Grade 3 and grade 4 neutropenia occurred following 53 % and 19 % of treatments, respectively [98]. Myelodysplastic syndrome and acute myeloid leukemia have also been reported after multiple infusions of high-dose ^{131}I -MIBG [92]. Hypothyroidism has also been reported following ^{131}I -MIBG therapy and patients should be pretreated with inorganic iodine in order to block ^{131}I uptake by the thyroid (such as daily potassium iodide beginning 24 h prior to therapy and continuing for a total of 10 days) [97].

Ultratrace iobenguane ^{131}I is a high specific activity form of ^{131}I -MIBG which uses a labeling approach resulting in non-carrier-added radiolabeled MIBG. Because uptake by the norepinephrine transporter is a competitive process, the presence of cold (nonradiolabeled) MIBG can diminish the uptake of the radiolabeled ^{131}I -MIBG. The carrier molecule, which does not contribute to therapeutic response, is bioactive and can cause hypertension, nausea, and vomiting when administered in high doses. In animal models, the high specific activity of ^{131}I -MIBG has been shown to result in higher levels of radioactivity in target tissue, greater therapeutic efficacy, and less appreciable cardiovascular side effects compared to the carrier-added counterpart [99]. Ultratrace iobenguane ^{131}I has been used safely in humans [100] and is under evaluation as part of a phase II clinical trial designed to evaluate its effectiveness in patients with MPP. The primary outcome measure is reduction in antihypertensive medications with secondary measures of overall tumor response as well as safety, quality of life, and overall survival [101]. Preliminary results have been presented. In this study of 49 patients treated with the therapy, 16 (32 %) achieved at least a 50 % reduction in antihypertensive drug use (95 % CI: 16–47 %), and 17 (35 %) had a partial response according to RECIST. The patients' median survival duration was 36 months [102]. The most common adverse event was nausea, followed by thrombocytopenia and fatigue.

Targeted Therapy

Inactivating mutations of the *SDHB* gene predispose pheochromocytoma and paraganglioma patients to metastases, and as such, these mutations are an important clinical predictor of overall survival [10, 58]. Inactivation of the *SDHB* gene allows the activation of hypoxia-inducible genes and the stabilization of hypoxia-inducible factor 2 α , which leads to the activation of the vascular endothelial growth factor (VEGF) gene, *VEGF*, the platelet-derived growth factor β (PDGFB) gene, *PDGFB*, and other genes associated with increased angiogenesis, abnormal cell growth, and decreased apoptosis [10, 103, 104]. Molecular targeted therapies that could modulate or inhibit the mechanisms underlying these and other hallmarks of the disease, including its mammalian target of rapamycin (mTOR) pathway activation and characteristic hematogenous and lymphatic spread to distant organs such as the bones, lungs, and liver, warrant investigation, especially in light of the fact that MPP response to conventional chemotherapy is poor.

Whether mTOR inhibitors such as everolimus offer a benefit in MPP patients is not clear [10, 105–107]; for example, in a case series that included 11 MPP patients, the mTOR inhibitor everolimus did not have an obvious benefit (NCT01152827). However, several tyrosine kinase inhibitors have shown some potential clinical benefit in previous studies, including case reports, case series, and a retrospective study, and are being investigated in prospective clinical trials.

Imatinib

Imatinib is a multityrosine kinase inhibitor of PDGFB receptor, stem cell factor, bcr-abl, and c-KIT. Imatinib has antitumor activity and it is approved for the treatment of chronic myeloid leukemia, GIST, dermatofibrosarcoma protuberans, myelodysplastic/myeloproliferative syndrome, aggressive systemic mastocytosis, hypereosinophilic syndrome, chronic eosinophilic leukemia, and refractory Philadelphia chromosome positive acute lymphocytic leukemia. Imatinib did not have a clinical benefit in the few patients with MPP treated with the drug [12, 108].

Sunitinib

Sunitinib is a multityrosine kinase inhibitor of VEGF receptor (VEGFR)-1, VEGFR-2, PDGFB receptor, RET, and c-KIT. Sunitinib has antitumor and antiangiogenic activity and it is approved for the treatment of kidney cancer, pancreatic neuroendocrine tumors, and gastrointestinal stromal tumors [10]. Two large referral cancer centers in the United States and Europe have published their experience with sunitinib in a retrospective, intention-to-treat study [12]. Of the 17 patients with progressive MPP who were included in the study 50% carried *SDHB* mutations, most had disease that was not responsive to CVD chemotherapy and/or MIBG, and 8 (47%) had disease with a positive radiographic response by RECIST 1.1 criteria [107]. The patients with responsive disease who were evaluated by ^{18}F -FDG PET had tumors that showed a substantial reduction of glucose uptake. Furthermore, 14 patients with baseline hypertension due to catecholamine excess had better blood pressure control after receiving treatment; 6 were able to maintain normal blood pressure with fewer and smaller doses of antihypertensives compared with their baseline antihypertensive use, and two patients discontinued antihypertensives altogether. The median overall survival and progression-free survival durations were 26.7 and 4.1 months, respectively. The brevity of the progression-free survival duration could be attributed to three patients discontinuing sunitinib early because of side effects that included the exacerbation of constitutional symptoms such as pain and fatigue and/or symptoms of catecholamine excess. In addition, several patients' tumors developed resistance to sunitinib. The longest reported response to sunitinib was of 36 months. The results of this study suggest that sunitinib could benefit some patients with progressive MPP, including patients whose disease is resistant to chemotherapy and MIBG irrespective of *SDHB* mutations. However, successful treatment with sunitinib requires that constitutional symptoms such as pain and fatigue and symptoms of catecholamines excess must be addressed before sunitinib is given.

A possible response to sunitinib has led to two ongoing phase II clinical trials of sunitinib in MPP patients. The NCT00843037 study is evaluating the daily use of 50 mg of oral sunitinib, 50 mg on a 4-weeks-on, 2-weeks-off schedule, and the FIRSTMAPP study is evaluating the daily use of 37.5 mg of oral sunitinib.

Pazopanib

Pazopanib, a multityrosine kinase inhibitor of VEGFR-1, VEGFR-2, VEGFR-3, c-KIT, and PDGFB receptor. Pazopanib is approved for the treatment of kidney cancer, and a phase II clinical trial is evaluating the use of the drug with escalation doses from 400 to 800 mg daily (NCT01340794). The agent is of special interest in the field of MPP because kidney cancer patients treated with pazopanib tolerated it better than sunitinib, exhibiting less intense and a lower rate of side effects. However, because many MPP patients have an increased, abnormal secretion of catecholamines, any pazopanib-induced tumor destruction may be associated with a dramatic release of catecholamines and the exacerbation of hormonal symptoms and drug-related side effects (e.g., hypertension).

Cabozantinib

Cabozantinib, a multityrosine kinase inhibitor of VEGFR1, VEGFR2, RET, and c-MET, is approved for the treatment of medullary thyroid and kidney carcinomas. Cabozantinib is being evaluated for the treatment of MPP patients in a prospective clinical trial (NCT02302833). Two common characteristics of MPP, bone metastases and angiogenesis, led to this clinical trial. About 80 % of MPP patients have bone metastases, which are usually lytic and are associated with increased morbidity and decreased overall survival [4]. Up to 80 % of MPP patients with bone metastases develop skeletal-related events such as bone pain, fractures, and cord compression. Once bone metastases occur, patients experience skeletal-related events rapidly; the median time from bone metastasis detection to skeletal-related event is 4.4 months. One study of bone metastases in MPP patients suggested that molecular targeted therapies could decrease

these patients' rates of skeletal-related events [4]. Cabozantinib has shown effectiveness in patients with skeletal metastases from cancers including lung, breast, and renal clear cell carcinomas.

Like sunitinib and pazopanib, cabozantinib is a potent inhibitor of angiogenesis. Tumor progression and resistance to sunitinib may be related to the compensatory activation of molecular pathways that are not inhibited by cabozantinib, such as the c-MET pathway. c-MET and VEGFRs cooperate to promote tumor angiogenesis, and MET upregulation may occur in response to VEGF pathway inhibition, thereby leading to tumor treatment resistance and growth. MET activation is a common feature of human tumors and frequently occurs in patients with bone metastases. Therefore, a clinical trial of cabozantinib in MPP patients seems promising.

Compared with sunitinib, pazopanib, and axitinib, cabozantinib may be a more promising agent for MPP patients. In addition to having antiangiogenic properties, the drug causes c-MET inhibition, which may be associated with decreased tumor growth, metastasis, bone metastases, and treatment resistance.

Immunotherapy

Although untested, immunotherapy may be a viable treatment option for MPP patients. Recent randomized phase III clinical trials have clearly demonstrated that patients with clear cell kidney cancers, the pathophysiologic mechanisms of which overlap with those of MPP, benefit from agents that regulate the programmed death 1 (PD-1)/programmed death ligand 1 immune pathway, indicating a potential role for the treatment in MPP patients. In kidney cancer patients, PD-1 activation is associated with decreased overall survival and poor prognosis, and in patients with pancreatic neuroendocrine tumors, PD-1 expression is associated with decreased survival [109]. An upcoming prospective phase I study of pembrolizumab will assess the expression of the PD-1 pathway and its response to immunotherapy in MPP patients.

Owing to PD1/PDL-1 activation, several tumors are able to avoid recognition and damage by the immune system [109–111]. Under normal conditions, natural killer cells recognize, attack, and destroy cancer cells. Macrophages and dendritic cells capture and process the cancer cell fragments, secrete numerous cytokines, and present tumor cell-derived antigens to T and B lymphocytes. The activation of these cells leads to the production of additional cytokines that promote the activation and recruitment of T cells and the synthesis of tumor cell-specific antibodies. These steps in turn lead to the elimination of the remnant tumor cells and the development of immune memory to prevent tumor recurrence [111].

Treatment of Hormonal and Tumor Burden-Related Complications

MPP patients may have a prolonged survival and during this time are exposed to the effect of catecholamine excess and complications associated with this excessive hormonal burden. Also, bone metastases may complicate the quality of life of MPP patients and deserve a special attention. In the following section, a suggested management of the long-term complications of MPP is provided.

Hypertension and Cardiovascular Disease

Complete resection of pheochromocytoma and paraganglioma is the best way to control and cure the hypertension and catecholamine excess associated with the disease [112]. However, surgical resection is often not curative for patients with MPP, and these patients require pharmacological intervention to control hypertension and prevent cardiovascular disease.

Blood pressure is usually managed with a combination of alpha- and beta-blockers. Treatment should start with a selective alpha-blocker such as doxazosin, terazosin, or prazosin or a nonselective alpha-blocker such as phenoxybenzamine. In set-

tings with limited resources, prazosin can be used; however, prazosin has a shorter half-life (~3 h) than doxazosin and terazosin do (~24 h) and is associated with more intraoperative fluctuations than phenoxybenzamine [113]. In candidates for surgical resection, phenoxybenzamine may be associated with a higher rate of side effects compared with other alpha blockers. Phenoxybenzamine has a prolonged half-life (~10 days) because of its irreversible binding to the alpha receptors and is thus frequently associated with a higher rate of sustained postoperative hypotension; because selective alpha blockers have a shorter half-life, they are rarely associated with this adverse event. In MPP patients, phenoxybenzamine may be an alternative for the long-term control of blood pressure; however, our practice uses routinely doxazosin in increasing doses until target blood pressure is achieved. Once blood pressure is normalized or if reflex tachycardia occurs, a beta blocker may be added. A useful alternative is a calcium channel blocker such as nifedipine or amlodipine as a complement to the alpha antagonism. In some patients unable to tolerate alpha blockade, calcium channel blockers are a useful alternative. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers could also be used alone or in combination with the aforementioned medications to treat hypertension and prevent cardiovascular events.

Catecholamine crisis is a potential complication of surgery, chemotherapy, and molecular targeted therapies. Patients with a catecholamine crisis are at risk for cardiovascular and gastrointestinal events. Before surgical or systemic oncologic therapy is offered, patients must have adequate blood pressure control, and their hearts must be protected with beta blockers. If a catecholamine crisis occurs, several medications can be used to control it. Some of the drugs used to treat hypertensive crises in pheochromocytoma patients are listed in Table 15.2.

Constipation

Constipation has been defined classically as fewer than 3 bowel movements per week, but the Rome III criteria for defining constipation also

Table 15.2 Drugs for the treatment of hypertensive crises in patients with pheochromocytoma

Drug/description	Preparation	Dose	Clinical aspects
Nitroprusside/direct arterial vasodilator	50 mg in 250 ml of D5%W	0.5–5 µg/kg/min, IV	Effect onset at 30 s, peak at 2 min. Do not use for >24 h at doses >5 µg/kg/min. Causes hypotension, tachycardia
Nicardipine/calcium channel blocker	25 mg in 250 ml of D5%W	Loading dose of 1–2.5 mg over 1–2 min, then 5–15 mg/h, IV	Controls tachycardia; may prevent catecholamine coronary-induced spasm
Magnesium sulfate/vasodilator	40 g in 500 ml of Ringer's solution	Loading dose of 1–2 g, then 1–3 g/h, IV	Causes muscle weakness and hypermagnesemia
Fenoldopam/dopamine 1 receptor agonist	10 mg in 250 ml of NS	0.1–1.6 µg/kg/min, IV	Renoprotective; causes tachycardia
Phentolamine/alpha-blocker	100 mg in 500 ml of D5%W	Bolus 5–15 mg, IV Infusion 0.2–2 mg/min, IV	Difficult to obtain
Esmolol/beta blocker	2.5 g in 250 ml of NS	Loading dose of 500 µg/kg over 1 min, then 50–300 µg/kg/min, IV	Controls tachycardia; use after initiation of a vasodilator

include straining, hard stools, sensation of incomplete evacuation, sensation of anorectal obstruction, and the necessity of manual maneuvers to facilitate defecation [114]. Although constipation can negatively affect MPP patients' quality of life, the complication is often overlooked and quite difficult to manage once it becomes severe [86].

There are very few reported cases of MPP with intestinal pseudo-obstruction [115–117] or bowel perforation [118–120]. The management of constipation includes a high-fiber diet with frequent water intake, 1 or 2 tablets of sennosides (senna) per day, and different types of laxatives depending on the severity of the symptom. This treatment must be adjusted accordingly for MPP patients with severe constipation (grade 3 or 4 constipation according to the Common Terminology Criteria for Adverse Events). The use of the Bisanz algorithm is recommended for patients with pheochromocytoma and paraganglioma who have constipation [86, 121]. Alpha- and beta-blockers do not improve constipation, as they do not increase motility or increase hydration. Theoretically, because diarrhea is one of the side effects associated with metyrosine [122], this drug might help improve constipation; however, our experience suggests that metyrosine is not very effective against constipation. A proto-

col for the management of uncomplicated constipation is presented in Table 15.3.

Skeletal-Related Events

MPP affects the bone in 71 % of patients [4], mostly in the form of lytic metastases that predominantly involve the spine, followed by the sacrum and pelvis, the proximal and distal long bones, and the skull. Among patients with neuroendocrine tumors, the prevalence of bone metastasis is 12 %, and of all neuroendocrine tumors, pheochromocytoma is the one that most frequently presents with bone metastases [123]. The complications of bone metastases include severe pain [124], fractures, and spinal cord compression. Hypercalcemia is uncommon. Bone metastases can also limit patients' mobility, range of movements, ability to perform daily life activities, and quality of life [125], which are important to conserve, as the treatment of MPP is often not curative.

No clinical guidelines or randomized clinical trials address the treatment of bone metastases. Retrospective studies indicate that bone pain may be treated with radiotherapy and antiresorptive medications such as denosumab or zoledronic acid. Monthly denosumab (120 mg) is an

Table 15.3 Protocol for the prevention and management of uncomplicated constipation in patients with malignant pheochromocytoma and paraganglioma

1. Rule out common causes of constipation, including dehydration, hypercalcemia, hypokalemia, hypothyroidism, low fiber intake, use of opioid analgesics, antiemetics, and/or oral iron
2. Increase daily oral fluid intake to at least 2 l
3. Encourage consumption of hot beverages such as coffee
4. Increase patient activity
5. Establish toilet schedule; stimulate morning gastrocolic reflex by regularly sitting on the toilet in the morning for at least 20 min
6. Regulate food intake with at least three daily meals to facilitate normal peristaltic pushdown
7. Increase fiber intake to 25–40 g in association with high fluid intake; the intake of fiber without water will constipate the patient
8. Use stimulant laxatives and stool softeners such as senna and docusate
9. Patient may use 1 or 2 tablespoons of mineral oil, vegetal oil, or magnesium hydroxide at bedtime
10. If there is no improvement, oral or rectal enemas with agents such as polyethylene glycol may be used

approved treatment for the prevention of the skeletal-related events associated with bone metastasis from solid tumors. This approval was based on three clinical trials [126–128]; the most relevant one included patients with solid tumors [126] other than breast and prostate cancer. In that study, denosumab was not inferior to zoledronic acid in preventing skeletal-related events. In another study, denosumab prolonged the time until the appearance of the first skeletal-related event by 6 months [129]. Our experience with MPP suggests that patients treated with denosumab have more improvement in their bone pain than those treated with zoledronic acid, with the additional advantage that denosumab requires neither intravenous infusion nor adjustment of the dose in patients with kidney failure.

If bone metastases are in a critical location, surgery, if feasible, is the first line of treatment, followed by radiation therapy [130, 131]. Bone-directed therapies might also be used. Patients may need more than one therapeutic strategy [123]. Patients must also undergo intense physical therapy to maintain a normal level of functionality.

Conclusions

The past 15 years have witnessed substantial progress in pheochromocytoma and paraganglioma in terms of genetics, diagnosis, and surgical approaches. However, our knowledge of MPPs remains very limited, and the development of effective systemic therapies for MPP patients has been very slow, and current guidelines describing the optimal treatment of patients with MMP are lacking. This is the most challenging aspect of this disease and one that requires more attention, as the overall survival of patients with MPP is worse than that of patients who do not have metastatic disease. However, several prospective clinical trials of novel and promising agents that target different mechanisms that may determine the aggressiveness of MPP are ongoing. Trials with agents such as Ultratrace ¹³¹I-MIBG, cabozantinib, sunitinib, and pembrolizumab are recruiting patients quickly, and their results will likely be presented within the next 2 or 3 years. In fact, the U.S. Food and Drug Administration has already granted Ultratrace ¹³¹I-MIBG a breakthrough therapy designation. Thus, a brighter future for MPP patients is in the horizon.

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